

Assessment of Variability in Stem Girth, Leaf Area, Leaf Thickness, Chlorophyll Content, and Their Correlation in Mango Hybrids

Homeshvari¹, S. I. Patil², B.P. Bisen³, S.K. Pandey⁴, Rajani Bisen⁵ and Bal Krishna^{2*}

DOI: 10.18811/ijpen.v11i03.12

ABSTRACT

The present study investigates the variability in stem girth, leaf area, chlorophyll content (total chlorophyll) and thickness of leaf in mango (*Mangifera indica* L.) hybrids derived from crosses involving two female parents Ratna and Kesar and five male cultivars: Totapuri (TP), Royal Special (RS), Kent (KN), Banganapalli (BP) and Tommy Atkins (TA). Significant genotypic differences were observed for all the traits evaluated. The hybrid from KS x BP showed the maximum median value (35 cm) for stem girth, followed by RT x KN and KS x RS (33 cm). For leaf area, hybrids from KS x TA (126.28 cm²) recorded the maximum median value, while maximum leaf area was recorded in hybrids derived from RT x TP (220.27 cm²). Hybrids KS x KN, RT x KN and KS x TP have median chlorophyll concentrations of 2.32, 2.28 and 2.27 mg g⁻¹, respectively. In RT hybrids, the highest median leaf thickness (0.37 mm) was observed in RT x BP, which is the same as KS hybrids, KS x TP (0.37 mm). Kesar-based hybrids generally outperformed for most of the parameters studied. The studies show a strong maternal influence on the hybrids. Further, the leaf area appears to be positively and significantly correlated with the stem girth. Total chlorophyll and leaf thickness are also positively and significantly correlated, suggesting a positive association with each other. The study highlights the potential of integrating physiological traits like stem girth, leaf area, chlorophyll content, and leaf thickness as early selection markers in mango breeding programs. These findings offer a basis for identifying parents and hybrids with enhanced vegetative vigor, with implications for genetic improvement and sustainable mango cultivation

Keywords: Mango hybrids, Stem girth, Leaf area, Chlorophyll content, Thickness of leaf.

Highlights

- There is a positive correlation between leaf area and stem girth in mango hybrids.
- This study shows that in mango hybrids, total chlorophyll content is positively and significantly correlated with the thickness of the leaf.
- The study has brought out that a female parent could influence leaf thickness, leaf area and chlorophyll content.

International Journal of Plant and Environment (2025);

ISSN: 2454-1117 (Print), 2455-202X (Online)

INTRODUCTION

Mango (*Mangifera indica* L.) is widely recognized as one of the most economically and culturally significant fruit crops in tropical and subtropical regions. Native to South Asia, it is now cultivated across the globe with India, China, Thailand, Mexico and the Philippines among the leading producers. India alone contributes to over 40% of global mango production. Mango is a vital crop not only due to its economic returns but also for its nutritional value, offering a rich source of vitamins A and C, antioxidants and dietary fiber. Sinha *et al.*, (2018) emphasized the need to consider both floral and vegetative traits in hybrid evaluation, especially leaf morphology as it affects not just photosynthesis but also tree architecture and reproductive success. Plant productivity largely depends on photosynthesis, which in turn is influenced by morphological and physiological traits of the plant. Among these, chlorophyll content and leaf area are central components in determining a plant's growth rate and photosynthetic output. Chlorophyll, the green pigment found in chloroplasts, absorbs light energy that drives the photosynthetic process, facilitating the conversion of carbon dioxide and water into carbohydrates and oxygen (Arnon, 1949). The total chlorophyll concentration, which includes chlorophyll a and chlorophyll b, directly reflects the photosynthetic capacity and general health status of the plant. Leaf area, on the other hand, represents the surface available for capturing sunlight

¹Department of Horticulture (Fruit Science), College of Agriculture, JNKVV, Jabalpur, M.P., India.

²Jain Research and Development, JISL, Jain Hills, Jalgaon, MH, India.

³Department of Horticulture, College of Agriculture, JNKVV, Jabalpur, M.P, India.

⁴Professor and Head, Department of Horticulture, College of Agriculture, JNKVV, Jabalpur, M.P., India.

⁵AICRP Sesame and Niger (ICAR), JNKVV, Jabalpur, M.P., India.

***Corresponding author:** Bal Krishna, Jain Research and Development, JISL, Jain Hills, Jalgaon, MH, India, Email: bal.krishna@jains.com

How to cite this article: Homeshvari., Patil, S. I., Bisen, B.P., Pandey, S.K., Bisen, R., Krishna, B. (2025). Assessment of Variability in Stem Girth, Leaf Area, Leaf Thickness, Chlorophyll Content, and Their Correlation in Mango Hybrids. *International Journal of Plant and Environment*. 11(3), 547-556.

Submitted: 17/05/2025 **Accepted:** 17/06/2025 **Published:** 30/09/2025

and is crucial for gas exchange and transpiration. A larger leaf area allows for greater interception of solar radiation and consequently higher photosynthetic rates, provided the plant can support the increased energy demands and water loss. Conversely, in stress-prone environments, plants with smaller

or more efficient leaf structures may exhibit better adaptation (Kaur *et al.*, 2024). Thus, the interaction between chlorophyll content and leaf area provides a composite view of the plant's physiological competence.

Chlorophyll content is one of the most widely used indicators in physiological studies due to its association with photosynthetic efficiency, nitrogen status, and general plant health. Chlorophyll *a* is the primary pigment involved in the light-dependent reactions of photosynthesis, while chlorophyll *b* acts as an accessory pigment that expands the range of light wavelengths the plant can utilize. The ratio of chlorophyll "*a*" and "*b*" also influences the efficiency of the light-harvesting complex in chloroplasts (Lichtenthaler *et al.*, 1981). Positive correlation between leaf area index, canopy volume, and fruit yield in mango (Samant *et al.*, 2020). For instance, hybrids with higher total chlorophyll per unit area tend to exhibit enhanced carbon fixation, greater biomass accumulation, and improved adaptability to fluctuating environmental conditions. Chlorophyll measurements also serve as early indicators of stress or nutrient deficiency, especially nitrogen, which is a key component of chlorophyll molecules. Modern techniques such as SPAD chlorophyll meters have made it easier to screen large populations for chlorophyll content, providing breeders with an efficient tool to identify superior genotypes at early growth stages. Additionally, chlorophyll traits are relatively stable across environments, enhancing their reliability as selection markers in breeding programs (Niaz *et al.*, 2024).

Leaf area is another vital trait that influences plant growth through its role in light capture, carbon assimilation, and water use. In mango, leaf morphology varies significantly across cultivars and is influenced by both genetic and environmental factors. Hybrids with larger leaves are generally more vigorous, with the capacity for higher photosynthetic rates and better canopy development. However, an excessively large leaf area can also lead to higher transpiration losses, especially in arid or semi-arid conditions, suggesting the need for an optimal balance depending on the agro-climatic zone (Kaur *et al.*, 2024). In previous research, varieties like Tommy Atkins and Totapuri were observed to possess larger leaf areas and higher chlorophyll contents, contributing to superior photosynthetic performance and productivity potential. This highlights the importance of evaluating both traits simultaneously in hybrid performance studies. Leaf area measurements, when combined with chlorophyll data provide a more comprehensive understanding of the photosynthetic dynamics of the plant. Moreover, leaf area distribution within the canopy affects light penetration and fruit development, making it a critical factor not only for physiological studies but also for orchard management.

Genetic diversity within mango varieties offers significant opportunities for selecting and developing hybrids with desirable agronomic and physiological traits. Crossbreeding among elite varieties allows for the recombination of favourable traits such as disease resistance, fruit quality, and photosynthetic efficiency. The choice of parental lines plays a pivotal role in determining the success of hybrid combinations.

Keeping in view the above, the present study has focused on hybrids developed from two commonly used female parents-Ratna and Kesar-crossed with five male cultivars: Totapuri, Royal Special, Kent, Banganapalli and Tommy Atkins. Ratna is known

for its moderate vigor and good fruit quality, while Kesar is widely appreciated for its flavor, productivity and adaptability. These two female lines offer contrasting physiological traits, thereby providing a valuable basis for hybridization. The male cultivars have other desirable qualities such as regular bearing (Totapuri and Banganapalli), off-season bearing (Royal Special), fruit size and fruit colour (Kent and Tommy Atkins). Previous studies have reported that hybrids from these parental combinations show significant variability in chlorophyll content and leaf area (Niaz *et al.*, 2024; Kaur *et al.*, 2024), besides other desirable characteristics in the hybrid population. Understanding the physiological differences is essential for the selection of hybrids that combine high photosynthetic efficiency with desirable horticultural characteristics.

Despite extensive research on mango varietal improvement, limited attention has been given to evaluating hybrids based on physiological traits such as chlorophyll concentration and leaf area. Given their critical roles in plant growth and yield determination, these traits can serve as early selection markers in mango breeding programs. The present study was designed to assess and compare the stem girth, leaf area, total chlorophyll content and leaf thickness across a range of mango hybrids involving Ratna and Kesar as female parents.

This physiological evaluation, backed by detailed statistical analysis, will contribute to a better understanding of the genetic and functional basis of productivity traits in mango. The findings may also help in identifying superior hybrids and parents with greater adaptability, resource-use efficiency, and yield potential under diverse agroecological conditions.

MATERIALS AND METHODS

Experimental Site and Plant Material

The study was conducted six-year-old Ultra High-Density Plantation (UHDP) during the 2022-23 to 2023-24 at the Research and Development Experimental Farm at Jain Hills, Agri Park of Jain Irrigation System Ltd., at Jalgaon, Maharashtra, India. The mango (*Mangifera indica* L.) hybrids developed from two female parental lines, Ratna (RT) and Kesar (KS), crossed with five male parental cultivars: Totapuri (TP), Royal Special (RS), Kent (KN), Banganapalli (BP) and Tommy Atkins (TA). A total of ten cross combinations and 757 hybrids (6 years old) were evaluated, along with their respective 6-year-old parents. The complete list of parental lines and hybrids is presented in (Table1). A hybrid population was planted in 2018 at a spacing of three meters between rows and two meters within rows, under Ultra High-Density Plantation and the orchard was managed as per Chaudhari *et al.*, (2017).

Stem girth (cm) measurement

Stem girth was measured at 10 cm height from the ground level with the help of a measuring tape and expressed in centimetres (cm). Plant stem girth is known to reflect the growth potential in fruit crops (Plavcova *et al.*, 2022).

Leaf area measurement

Leaf area was determined using a CI-203 Handheld Laser Leaf Area Meter (CID Bio-Science Inc., USA). For each genotype, ten

Table 1: Parental combinations and mango hybrids used in the study

Parent (as a female)	Parent (as a male)	Hybrids	No. of hybrids
Ratna (RT)	Totapuri (TP)	RT x TP	259
	Royal Special (RS)	RT x RS	121
	Kent (KN)	RT x KN	81
	Banganapalli (BP)	RT x BP	73
	Tommy Atkins (TA)	RT x TA	51
Kesar (KS)	Totapuri (TP)	KS x TP	52
	Royal Special (RS)	KS x RS	29
	Kent (KN)	KS x KN	50
	Banganapalli (BP)	KS x BP	18
	Tommy Atkins (TA)	KS x TA	23

fully expanded leaves were randomly selected and measured. The average leaf area per parental variety and its hybrids was recorded in cm².

Chlorophyll content estimation

Extraction and chlorophyll estimation were performed following the method described by Arnon (1949). Chlorophyll was extracted in acetone (80%) from a five-gram fresh leaf sample and the absorbance was measured at 663 and 645 nm. Additionally, to take care of the background turbidity of the sample, absorbance was also measured at 750 nm.

Leaf thickness (mm) measurement

The thickness of the leaf was measured by using the Mavotank Thickness Gauge. Ten leaves were measured and an average of ten was calculated and recorded in mm.

Statistical analysis

The boxplots were generated using R software package 'qboxplot' version 0.2 (Pike, 2022) in R Studio to visualize the distribution and variability of stem girth, leaf area, total chlorophyll content, and thickness of leaf among mango hybrids and their parental genotypes. The ggplot2 package was employed for creating the plots (Gomez-Rubio, 2017). Boxplots were used as it is an effective tool for identifying medians, interquartile ranges (IQR) and potential outliers within datasets, enabling comparative assessment of traits across genotypes.

The correlation was estimated by Pearson's coefficient using Pearson's linear correlation in the R package using 'correlation' (Makowski *et al.*, 2020).

RESULTS

Stem girth, leaf area, chlorophyll contents and thickness of leaf: Stem girth of parent varieties ranged from 33 to 40 cm. Maximum stem girth was observed for variety Kent (40 cm) followed by Ratna (38 cm) and Kesar (39 cm), minimum stem girth was recorded for variety Tommy Atkins. Totapuri, Royal Special and Banganapalli recorded similar stem girth 35, 35 and 36 cm, respectively. Evaluation of leaf area showed that Totapuri has the highest mean leaf area (129.25 cm²), suggesting a higher surface area for light interception and gas exchange. This was followed by Tommy Atkins (118.02 cm²) and Banganapalli (116.70 cm²), whereas the smallest leaf area (83.02 cm²) was observed in Royal Special (Table 2). These variations underline the physiological diversity among mango varieties, with implications for their photosynthetic capacity and productivity potential. The chlorophyll content and leaf area across seven mango varieties revealed significant variability among varieties. Tommy Atkins exhibited high total chlorophyll (3.24 mg/g) content, followed by Ratna (2.65 mg/g) and Totapuri (2.29 mg/g), indicating significant variation among the parents used for hybridisation. In contrast, Banganapalli recorded the lowest total chlorophyll content at 1.47 mg/g. The thickness of the leaf also ranged from 0.36 to 0.47 (mm). The highest leaf thickness was recorded in Kesar (0.47 mm) and the lowest leaf thickness was recorded in Tommy Atkins (0.36 mm) followed by Ratna (0.37 mm). The thickness of the leaf was similar in Totapuri, Royal Special, Kent and Banganapalli (0.42, 0.41, 0.42 and 0.44, respectively).

The data pertaining to stem girth (cm) among the ten different mango hybrids are presented graphically in the form of a boxplot (Fig. 1). A wide range of variation was observed, indicating significant genetic diversity among the hybrids with respect to vegetative growth, as reflected by stem girth. Among all the hybrids derived from Ratna female parent, RT x KN recorded the highest median stem girth of 33 cm, followed by RT x TP, RT x RS and RT x BP (32 cm), respectively. RT x TA recorded a lower median value (30 cm). In contrast, KS x BP recorded the highest median (35 cm) followed by KS x RS (33 cm) and KS x TA, despite having a median stem girth of about 32 cm, which displayed the widest interquartile range (IQR), suggesting high internal variability among replications. KS x TP and KS x KN

Table 2: Stem girth (cm), leaf area (cm²), total chlorophyll content (mg/g) and leaf thickness (mm) of parents used for hybridization

Varieties	Stem girth (cm)	Leaf area (cm ²)	Total chlorophyll (mg/g)	Thickness of leaf (mm)
Ratna	38	91.83	2.65	0.37
Kesar	39	94.06	2.10	0.47
Totapuri	35	129.25	2.29	0.42
Royal Special	35	83.02	2.10	0.41
Kent	40	109.87	2.08	0.42
Banganapalli	36	116.70	1.47	0.44
Tommy Atkins	33	118.02	3.24	0.36
SE(±)	0.95	6.34	0.21	0.01

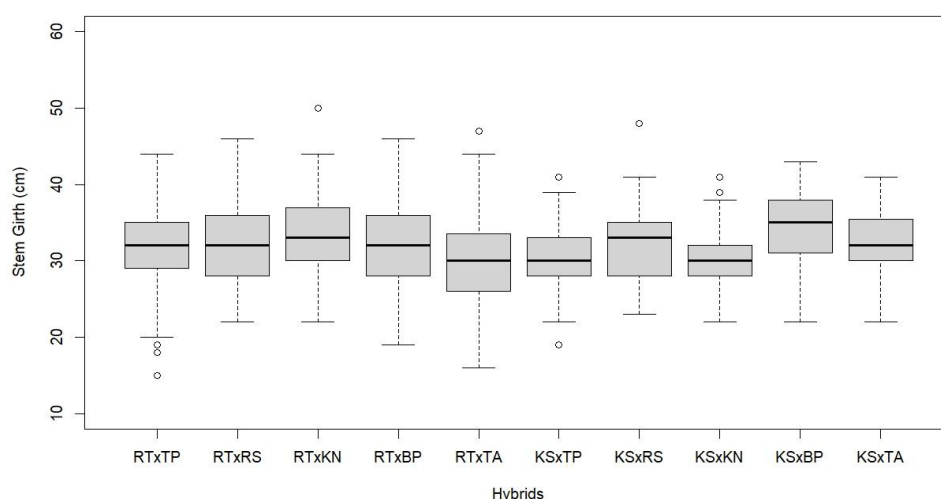


Fig. 1: Variation in stem girth among mango hybrids and parental combinations

showed lower stem girth medians of approximately 30.0 cm. Outliers were also evident in hybrids such as RT x TP, KS x TP, and KS x RS, where individual observations fell below or above the typical data range, as indicated by the extended whiskers. RT x TP, for instance, had minimum stem girth values falling near 15-19 cm, while its upper limit extended. Similarly, KS x TP which, displayed an outlier at the lower end (19 cm), but also reached up to nearly 41 cm, showcasing a diverse plant response within the same hybrid group.

Leaf area varied significantly among the 10 mango hybrid combinations (Fig. 02). Combination RT x TP and KS x TA resulted in hybrids with a wide range of leaf area, whereas KS x BP and KS x RS progenies had a narrow range of leaf area variation. The other combinations resulted in between these.

The hybrids of Ratna showed moderate to low variability in leaf area. Among them, RT x TP exhibited the highest maximum leaf area of 220.27 cm² but this appears as an outlier, as the median values in this group ranged from 82.92 cm² (RT x KN) to 100.87 cm² (RT x TP), suggesting an overall moderate leaf size distribution. Minimum values in these

hybrids ranged from 48.61 cm² (RT x KN) to 58.74 cm² (RT x TA), indicating a generally smaller baseline. In contrast, the hybrids of Kesar showed consistently larger leaf area values. KS x TA had the highest median (126.28 cm²) along with a minimum and maximum range of 54.37 to 179.78 cm² values among all hybrids, followed closely by KS x BP and KS x KN with median values of 115.7 cm² and 92.65 cm², respectively. Additionally, this group showed higher 3rd quartile values, reflecting a general trend toward larger leaves across the upper range. The minimum values in this group were also relatively higher, particularly in KS x BP (80.78 cm²), which suggests more uniformly larger leaves.

Hybrids RT x TA, KS x TP and KS x KN had median leaf areas ranging between 91.68 cm² and 95.50 cm², indicating relatively moderate leaf size. Hybrids RT x RS, RT x KN and RT x BP showed relatively smaller leaf areas, with median values between 82.92 cm² and 90.96 cm². The smallest leaves were observed in hybrid KS x RS, which recorded the lowest median leaf area at 68.68 cm² and the lowest minimum value at 46.59 cm², indicating restricted leaf expansion.

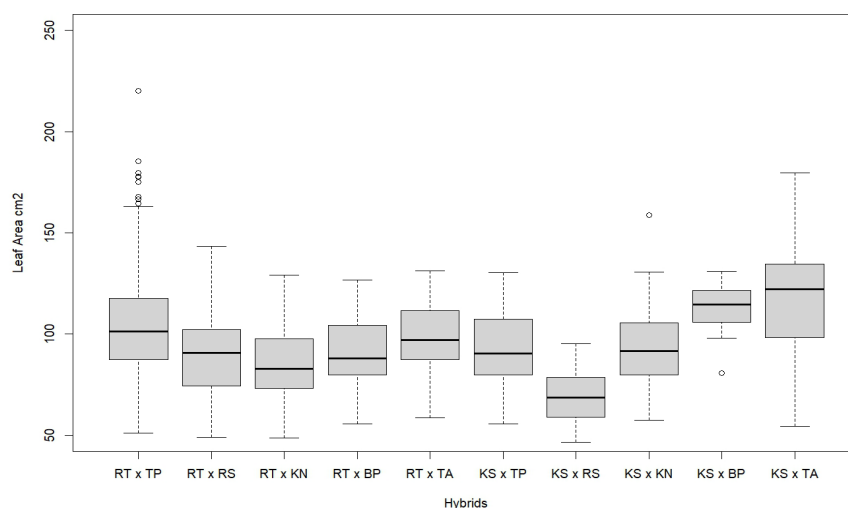


Fig. 2: Variation in leaf area among mango hybrids and parental combinations

Hybrids KS x TA and KS x BP stood out for their consistently large leaf areas across all statistical parameters compared to the Ratna hybrids, indicating potential genetic influence on leaf size traits, while hybrid KS x RS exhibited the most limited leaf development. These findings are important for identifying genotypes with superior canopy traits that may contribute to enhanced photosynthetic performance and yield potential in mango breeding programs. Overall, the Kesar parent line hybrids demonstrated that hybrids involving Kesar as a female parent tend to produce leaves with larger surface area, which may have implications for photosynthetic capacity and overall plant vigor.

The total chlorophyll (a+b) content across 10 mango hybrids cross combinations revealed considerable variation, highlighting the underlying genetic diversity among hybrids.

The Ratna as a female parental line, particularly hybrids RT x KN and RT x BP, showed relatively higher median chlorophyll values (2.28 and 2.25 mg/g, respectively), along with extended upper quartile ranges (2.46 and 2.45 mg/g), suggesting a concentration of higher chlorophyll levels. Hybrid RT x TP, however, showed the lowest minimum value (1.04 mg/g) and the widest variability, indicating inconsistent physiological performance. In contrast, among the Kesar as a female parental line, hybrids KS x KN and KS x BP showed strong performance with medians of 2.32 and 2.20 mg/g, and high third quartile values (2.47 and 2.42 mg/g). Notably, hybrid KS x TA recorded a high third quartile (2.49 mg/g) and maximum (2.85 mg/g), but with a relatively lower median (2.14 mg/g), implying broader within-genotype variation. (Fig. 3).

To better understand the variation and breeding potential in terms of total chlorophyll content, a comparative analysis was conducted between the hybrids of Ratna as a female parental line and the hybrids from Kesar as a female parental line. The results indicated noticeable differences in chlorophyll distribution patterns between the two groups, both in terms

of central tendency and variability. Boxplot visualization supported these observations, revealing moderate variability in most genotypes, with a few exceptions displaying wider spreads and potential outliers (notably hybrid KS x TP and RT x RS). Hybrid KS x RS showed the lowest median in this group (2.17 mg/g), on par with the hybrid RT x TP of the first group. Ranking of genotypes based on median values showed hybrid KS x KN as the top performer, followed by hybrids KS x KN, KS x TP and KS x BP (Fig 03). These differences in chlorophyll content have important breeding implications. Higher total chlorophyll content is often positively correlated with higher photosynthetic rate, biomass accumulation, and ultimately fruit yield. Therefore, hybrids exhibiting elevated and stable chlorophyll levels (e.g. RT x KN, RT x BP, KS x KN and KS x TP) are ideal hybrids for selection in breeding programs aimed at enhancing vegetative vigor and productivity.

Statistically, while the median values between both the female parental lines were relatively close, the Kesar parental line hybrids demonstrated slightly better upper quartile stability and fewer extremely low outliers. This may suggest a more consistent chlorophyll accumulation pattern in the latter group. However, hybrids RT x KN and RT x BP in the Ratna parental line outperformed several others in both quartile range and maximum values, which is indicative of their high potential for photosynthetic efficiency. Overall, the comparison reveals that both parental lines include promising hybrids, though the Kesar parental line shows greater consistency, while the Ratna parental line presents outliers with exceptionally high or low chlorophyll content.

Thickness of Leaf

The analysis of leaf thickness among various mango hybrids was presented in Fig. 04. A significant variation was observed across the hybrids, indicating the influence of parental combinations on anatomical traits like leaf thickness.

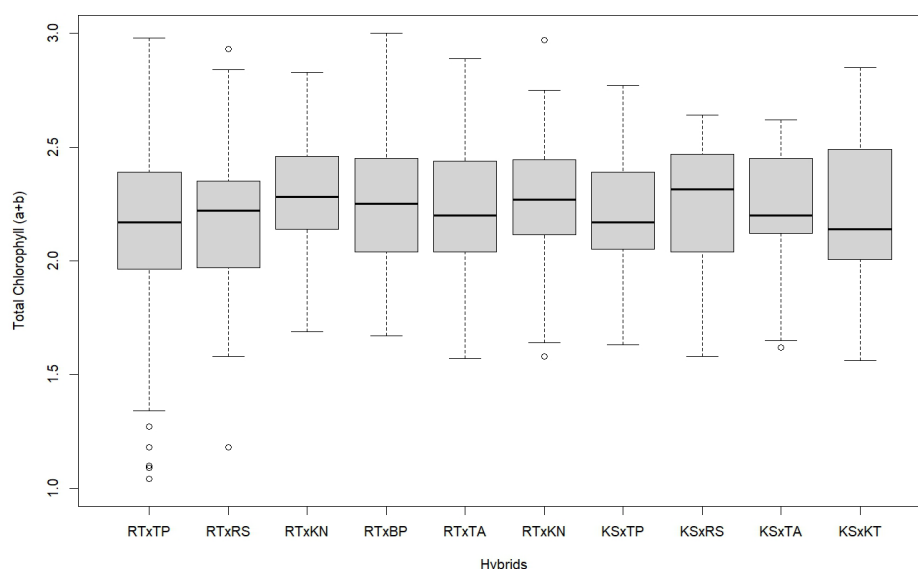


Fig. 3: Variation in total chlorophyll content among mango hybrids and parental combinations

The results revealed considerable variation in leaf thickness among the hybrids. In RT hybrids, the highest median thickness (0.37 mm) was observed in RT x BP, while RT x TA had the lowest median thickness (0.31 mm). The hybrids RT x TP, RT x RS, and RT x KN displayed similar trends with median thickness values ranging between 0.35 mm, indicating relatively less variation. On the other hand, among the KS hybrids, the maximum median leaf thickness (0.48 mm) was recorded in KS x BP, followed by KS x TP (0.37 mm). Hybrids under KS x RS, KS x KN and KS x TA showed similar median values (0.33, 0.34 and 0.34, respectively). These hybrids also showed a wider interquartile range, suggesting greater phenotypic variability. The presence of outliers in KS x KN further indicated genetic heterogeneity in leaf anatomical traits. The Kesar-derived hybrids (KS x TP, KS x RS, KS x KN, KS x BP, KS x TA) generally exhibited higher median values and broader ranges of leaf thickness than the Ratna-derived hybrids (RT x TP, RT x RS, RT x KN, RT x BP, RT x TA). These findings emphasize that parental selection significantly affects anatomical traits in mango hybrids. Kesar-based hybrids may offer better tolerance to environmental stresses due to thicker leaves, which could enhance photosynthetic efficiency and reduce transpiration. On the other hand, hybrids with thinner leaves, such as RT x TA and KS x KN, may require further physiological evaluation before being recommended for regions with harsh climatic conditions.

Correlation between Stem Girth and Leaf Area

The correlation analysis between stem girth and leaf area across multiple hybrid combinations revealed a consistent and statistically significant positive relationship (Table 3). This suggests that as the leaf area increases, the stem girth correspondingly increases. This indicates a mutually reinforcing relationship between foliage, responsible for carbon assimilation, and increased structural support.

The degree of association varied across hybrids, ranging from moderate to very strong. The highest correlation was found in the KS x TA cross (0.98**), indicating an almost linear relationship. This implies that in this cross, stem girth can serve as a strong predictor of leaf area, likely due to genetic synergy in vegetative growth traits. Other crosses like KS x RS (0.89**) and

RT x RS (0.86**) also showed strong positive correlations. Moderate correlations in crosses such as RT x BP (0.51**) and RT x TP (0.47**) imply that these hybrids may be influenced by more variable environmental or genetic interactions. Nevertheless, the statistically significant data across all hybrids reinforces the reliability of stem girth as a surrogate trait for predicting canopy development.

Correlation between Total Chlorophyll Content and Leaf Thickness

The correlation analysis between total chlorophyll content and leaf thickness revealed significant variability among different crosses (Table 3). The RT x KN combination showed a strong positive and significant correlation ($r = 0.76$), while the RT x BP and RT x TA crosses also exhibited significant correlations of 0.49 and 0.37, respectively. In the RT x TP cross, a positive and significant correlation was observed ($r = 0.35$), suggesting a moderate association between the two traits. However, in the RT x RS cross, the correlation was positive but non-significant ($r = 0.12$, NS), indicating a weak relationship. For the KS crosses, all showed positive and significant correlations. The KS x TP cross-correlated of 0.50, while KS x RS, KS x KN, and KS x BP showed stronger correlations of 0.68, 0.59, and 0.60, respectively. Notably, the KS x TA cross recorded the highest correlation value ($r = 0.95$), which was highly significant, indicating a near-linear relationship between leaf thickness and total chlorophyll content in this hybrid suggesting that the thickness of the leaf is almost directly proportional to total chlorophyll content in this combination.

Discussion

The present study underscores substantial physiological and morphological variability among mango hybrids derived from Ratna and Kesar female lines. Notably, the observed differences in stem girth, leaf area, total chlorophyll contents, and thickness of leaves affirm the genetic and environmental influence on these key physiological traits. These traits are fundamental for understanding hybrid vigor, growth dynamics, and potential productivity.

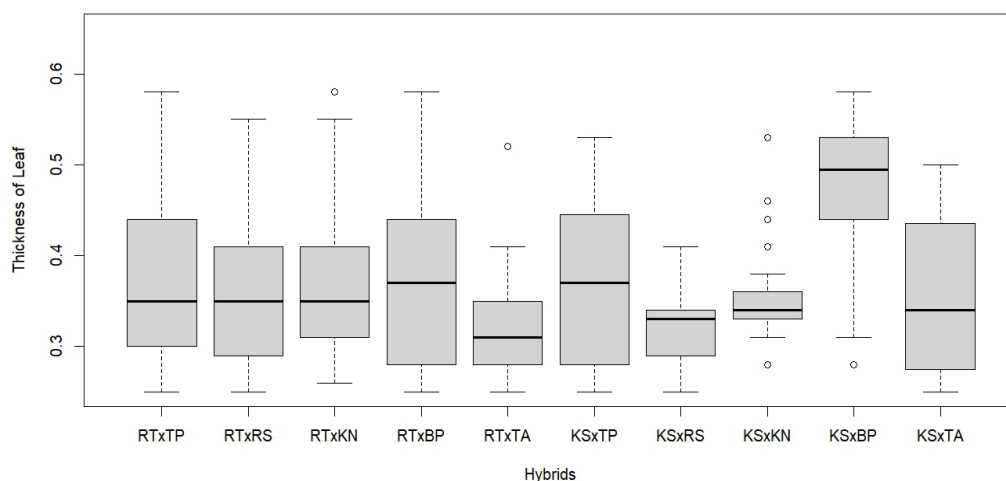


Fig. 4: Boxplot representing the distribution of the thickness of the leaf (mm) across various mango hybrids from different parental combinations

Table 3: Correlation between stem girth and leaf area and between total chlorophyll content and leaf thickness across different cross combinations in mango

Crosses	Stem girth and leaf Area	Total chlorophyll content and the thickness of the leaf
RT x TP	0.47**	0.35**
RT x RS	0.86**	0.12NS
RT x KN	0.58**	0.76**
RT x BP	0.51**	0.49**
RT x TA	0.81**	0.37**
KS x TP	0.75**	0.50**
KS x RS	0.89**	0.68**
KS x KN	0.58**	0.59**
KS x BP	0.72**	0.60**
KS x TA	0.98**	0.95**

p < 0.05: Statistically significant (marked with *), p < 0.01: Highly significant (marked with **) and NS: Non significant

Interestingly, KS x BP, in addition to having the highest median, also showed a relatively compact distribution of values (low variance) and absence of extreme outliers, highlighting it as a genetically stable and superior-performing hybrid in terms of vegetative stem thickness. This indicates that KS x BP might be a promising cross-combination for further evaluation and propagation, especially where plant vigor is a key trait of selection. According to Reddy and Singh (1991) and earlier works by Chacko *et al.*, (1982), robust vegetative growth-especially a thicker stem, is positively correlated with higher carbohydrate reserves, which in turn influence fruit set and development during alternate bearing cycles. The boxplot also revealed that hybrids derived from the RT parent line generally exhibited higher stem girth as compared to those from the KS parent line. This trend may reflect the superior combining ability of the RT parent concerning vegetative growth traits. These values suggest a stronger vegetative growth potential in these hybrids, possibly due to better genetic makeup and hybrid vigour. These findings are consistent with the observations of Majumder *et al.*, (2012), who reported that vegetative traits like plant height and stem development are significantly associated with fruit yield and can be used as indirect selection criteria in mango breeding.

Hybrids involving Kesar as a female parent, such as KS x KN and KS x TA displayed consistently high and uniform chlorophyll levels and leaf area, indicating the stable contribution of the Kesar parent in transmitting favourable traits. These findings are similar to those by Rymbai *et al.*, (2014) and Bhamini *et al.*, (2018), who both emphasized substantial morphological variation across mango germplasm due to inherent genetic diversity. The variability in our study reflects not only heterosis in hybrids but also the wide phenotypic plasticity present in mango. These findings mirror the work of Sridhar *et al.*, (2022), who documented significant morphological differences across mango cultivars, emphasizing the value of leaf traits in hybrid selection and varietal characterization. The broader and more

uniform leaf distribution in these hybrids is a favourable indicator for enhanced light capture and gas exchange, as highlighted by Schaffer and Gaye (1989), who linked high chlorophyll and leaf surface area with photosynthetic performance under optimal light exposure. Sinha *et al.*, (2018) also demonstrated variability in leaf characteristics like shape, size, and color between mango hybrids and parents, attributing this to differential gene expression and hybrid vigor. Moreover, complementing these findings, the work of Igbari *et al.*, (2019) and Jadhav *et al.*, (2022) affirmed that mango genotypes exhibit considerable diversity in leaf morphology, including leaf length, width, and shape, which are all vital for early selection and cultivar differentiation. Our results reinforce their findings, suggesting that selection of physiologically superior hybrids can begin as early as the vegetative stage using these indicators.

On the other hand, Ratna-derived hybrids like RT x BP and RT x KN also demonstrated commendable chlorophyll levels, albeit with greater variability. This broader range may be attributed to genotypic heterogeneity or environmental sensitivity, supporting the observations by Rani *et al.*, (2018), who found a significant decline in chlorophyll content under stress conditions like salinity or nutrient imbalance.

Environmental conditions, particularly temperature, light, and nutrient availability, influence chlorophyll biosynthesis and degradation. In this context, hybrids with higher and more stable chlorophyll levels-like those from Kesar crosses-may offer enhanced resilience under fluctuating agro-climatic conditions. Gadallah *et al.*, (2020) observed a reduction in chlorophyll content and fluorescence under cold stress, emphasizing that chlorophyll degradation is a reliable stress marker. Similarly, Rani *et al.*, (2018) demonstrated a strong link between stressful growing conditions (such as poor nutrition or disease pressure) and lowered chlorophyll concentration, which could be a precursor to malformation disorder. In our study, hybrids such as RT x TP showed wide variation and outliers in chlorophyll content, which may indicate environmental sensitivity or physiological instability. This reflects the need to not only consider high average values but also the consistency and stability of these traits when selecting for climate-resilient genotypes.

The link between leaf area and photosynthetic potential is another important outcome of this study. Hybrids like KS x TA and RT x TP had wide ranges and higher median values in leaf area, suggesting stronger vegetative vigor. These findings are similar to those of Samant *et al.*, (2020), who established a positive correlation between leaf area index, canopy volume, and fruit yield in mango. However, excessively large leaf areas could lead to higher transpiration losses in arid climates, indicating the importance of balancing leaf traits based on agro-ecological zones.

Chlorophyll content is a proxy for photosynthetic potential and general plant health. In our study, hybrids with higher chlorophyll levels often also had larger leaf areas, which may suggest better light absorption and higher assimilation rates. This trend supports conclusions drawn by Schaffer and Gaye (1989), who found that leaf chlorophyll and nitrogen levels directly affect gas exchange and carbon fixation in mango. Studies similar to those of Kaur *et al.*, (2024), can help unravel

the genetic regulation of leaf development and chlorophyll metabolism in mango. Combining physiological screening (as in this study) with molecular profiling could greatly enhance the precision of mango breeding, allowing the identification of gene markers associated with desirable traits such as higher chlorophyll synthesis, better leaf structure, and adaptive plasticity. The broader variability observed across hybrids can be traced to their diverse parental backgrounds. Morphological diversity and genetic divergence in mango cultivars, as reported by Rymbai *et al.*, (2014) and Bhamini *et al.*, (2018), provide valuable opportunities for selecting heterotic hybrids with favourable physiological traits.

In terms of measurement approaches, Ghoreishi *et al.*, (2012) demonstrated that non-destructive estimations of leaf area using simple linear models based on length and width are both accurate and cost-effective. This supports our methodology and affirms the utility of leaf area as a reliable index in mango physiological evaluation. Rich *et al.*, (1995) further extended this concept with the LAICALC software, which calculates Leaf Area Index (LAI) from gap fraction data and integrates leaf geometry to assess canopy structure-emphasizing the relationship between leaf traits and canopy photosynthetic capacity. From a remote sensing perspective, Niaz *et al.*, (2024) illustrated how chlorophyll content, when measured through SPAD meters and correlated with Normalized Difference Vegetation Index (NDVI) values from satellite imagery, provides valuable insights into crop health and yield forecasting. Their study revealed that orchards, especially mango, had higher chlorophyll content than field crops, reinforcing the role of leaf chlorophyll as a key indicator of physiological health. This is consistent with our findings, where higher chlorophyll content in certain hybrids corresponded with more vigorous vegetative expression as indicated by the stem girth.

Importance of leaf morphology for hybrid evaluation is emphasized by Sinha *et al.*, (2018). This multidimensional approach to hybrid selection strengthens the scope for more precise and early-stage screening. The practical implication of these findings is substantial. Since mango has a long juvenile phase, early-stage selection based on vegetative traits like chlorophyll content and leaf area can significantly shorten breeding cycles. Studies such as those by Kumar *et al.*, (2018) and Pike (2022) emphasize the importance of statistical and visual tools like quantile-based boxplots to analyze trait distribution and identify outliers. In our study, outlier detection in hybrids such as RT x TP underscores the need to consider not just mean performance but also variability and stability. In sum, this study validates chlorophyll content and leaf area as robust, early indicators of hybrid vigor and adaptability. When integrated with morphological, biochemical, and environmental sensitivity data from prior research, these traits provide a comprehensive framework for identifying superior hybrids. Such an approach enables mango breeders to optimize selection strategies for yield, resilience, and physiological efficiency across diverse agro-climatic zones.

In correlation analysis between stem girth and leaf area, these findings are supported by previous studies in mango, which emphasized the importance of leaf area for fruit development and overall productivity. Reddy and Singh (1991)

reported that mango fruit growth was positively influenced by leaf area and number, showing that a certain threshold of vegetative structure is required for optimal fruit development. Similarly, Singh *et al.*, (2007) highlighted that more than 30 leaves were required to support a single fruit's normal growth, with both current photosynthates and stored carbohydrates contributing significantly to fruit development. Chacko *et al.*, (1982) had concluded that a minimum leaf-to-fruit ratio is crucial and that reserve metabolites play a vital role during heavy bearing years. Moreover, the correlation between stem girth and leaf area has also been shown to contribute indirectly to yield traits. Majumder *et al.*, (2012) noted that although leaf area exhibited a negative direct effect on yield but it interacted indirectly with other yield-contributing traits, suggesting its complex role in overall plant productivity. These results clearly demonstrated that stem girth and leaf area are positively and significantly correlated across all crosses studied, with the strength of the correlation varying from moderate to very strong.

The crosses involving the RT parent exhibited more variability. While RT x KN showed a strong correlation ($r = 0.756^{**}$), RT x RS had a weak and non-significant correlation ($r = 0.123$ NS), indicating a potential genotype x environment interaction or limited additive genetic control over these traits. This is in line with findings by Indian *et al.*, (2022), who emphasized that low heritability of chlorophyll content suggests greater environmental influence and lower selection efficiency. Further support for these conclusions comes from studies on stress responses in mango leaves. Lucena *et al.*, (2012) demonstrated that salt stress significantly affected chlorophyll fluorescence and leaf morphology, indicating the importance of leaf anatomical integrity in maintaining photosynthetic function under adverse conditions. Similarly, Elsheery and Cao (2008) reported that under drought stress, mango cultivars with thicker leaves and higher chlorophyll content showed better physiological resilience and faster recovery upon rehydration. Jutamanee and Onnom (2016) revealed that mango leaves exposed to moderate light conditions maintained higher chlorophyll content and photosynthetic performance, emphasizing the adaptive significance of leaf structural traits like thickness in optimizing chloroplast distribution and light capture efficiency.

Conversely, highest correlation was observed in the KS x TA cross ($r = 0.953^{**}$), suggesting a near-linear relationship between leaf thickness and total chlorophyll content. This strong association highlights the potential of specific genetic combinations to enhance both anatomical and physiological leaf traits simultaneously suggesting that the thickness of the leaf is almost directly proportional to total chlorophyll content in this combination. Similar observations were reported by Urban *et al.*, (2003), who found that thicker mango leaves generally had higher nitrogen and chlorophyll content, contributing to improved photosynthetic capacity and carbon assimilation. In other KS-based crosses such as KS x RS, KS x KN, and KS x BP, the correlations ranged from moderate to strong ($r = 0.589$ - 0.683^{**}), further supporting the hypothesis that KS as a parental line contributes positively to both traits. These results are consistent with those reported by Esan *et al.*, (2024), who documented significant genetic variability among mango cultivars and

noted that traits like leaf thickness and chlorophyll content are often co-inherited and influenced by environmental and genetic factors.

CONCLUSION

The present study demonstrates that significant genetic variability exists among mango hybrids in terms of stem girth, leaf area, total chlorophyll contents and thickness of leaf, vital physiological traits closely linked to photosynthetic efficiency, vegetative vigor and potential adaptability. Hybrids derived from Kesar, particularly KS x KN and KS x TA, showed superior and more stable performance across all traits, suggesting that Kesar is a promising female parent for developing high-performing genotypes. The variation observed among Ratna-derived hybrids also indicates opportunities for targeted selection. These traits-being easy to measure, non-destructive, and highly indicative of plant health-can serve as early selection indices in mango breeding programs, reducing the long juvenile period typically required for fruit evaluation. Furthermore, when combined with insights from molecular biology, morphological characterization, and phytochemical profiling, the use of physiological markers such as chlorophyll and leaf area offers a comprehensive framework for selecting hybrids not only for agronomic excellence but also for nutritional and stress-resilience traits. In the light of these findings, stem girth, which can be easily measured and is less cumbersome than determining the leaf area, can be used as a valuable trait for early-stage selection in mango breeding programs aiming to enhance vegetative vigor and, by extension, reproductive performance. Selecting genotypes with superior stem girth may indirectly ensure better canopy development, improved photosynthetic efficiency, and greater potential for fruit yield. Crosses involving the KS parent generally exhibit stronger correlations between leaf thickness and chlorophyll content compared to those with RT as the parent, implying potential genetic influence from the KS line in enhancing both traits simultaneously. Thus, this study provides a valuable contribution toward the integration of physiological and genetic tools in mango improvement strategies, with implications for both commercial cultivation and future research.

ACKNOWLEDGMENT

Authors are thankful to Dr. B. H. Jain, Founder Architect, Jain Irrigation System Ltd. (JISL) and its management for initiating and funding of mango improvement program. Authors are also thankful to JISL management for providing fellowship to Miss Homeshvari and research facilities at Jain R&D.

AUTHOR CONTRIBUTION

BK and BPB conceived the research and planned the experiments, H and SIP conducted the experiments and gathered the data, and H and BK analyzed the data. H and BK wrote the manuscript. BPB, SKP and RB reviewed the data and manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

REFERENCES

- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. *Plant Physiology*, 24(1), 1–15. <https://doi.org/10.1104/pp.24.1.1>
- Bhamini, K., Kumar, A., Jaiswal, U. S., Ahmad, M. F., & Rani, R. (2018). Morphological characterization of mango (*Mangifera indica* L.) germplasm using DUS testing. *International Journal of Current Microbiology and Applied Sciences*, 7(5), 2944–2959. <https://doi.org/10.20546/ijcmas.2018.705.343>
- Chacko, E. K., Reddy, Y. T. N., & Ananthanarayanan, T. V. (1982). Studies on the relationship between leaf number and area and fruit development in mango (*Mangifera indica* L.). *Journal of Horticultural Science*, 57(4), 483–492. <https://doi.org/10.1080/00221589.1982.11515082>
- Chaudhari, A. U., Krishna, Badd, & Balasubrahmanyam, V. R. (2017). Standardization of a package of practices for mango cultivars 'Alphonso', 'Ratna' and 'Totapuri' under ultra-high-density planting. *Acta Horticulturae*, 1183, 187–194. DOI: 10.17660/ActaHortic.2017.1183.26
- Elsheery, N. I., & Cao, K. F. (2008). Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiologiae Plantarum*, 30(6), 769–777. <https://doi.org/10.1007/s11738-008-0179-x>
- Esan, V. I., Ogunbode, T. O., Ogunlaran, O. M., Ayegboyin, M. H., Omilani, O. O., Sangoyomi, T. E., & Akande, J. A. (2024). Genetic variability and morpho-agronomic characterization of some mango (*Mangifera indica* L.) cultivars and varieties in Nigeria. *International Journal of Fruit Science*, 24(1), 256–272. <https://doi.org/10.1080/15538362.2024.2389102>
- Gadallah, F. M., El-Yazal, M. A. S., Abdel-Samad, G. A., & Sayed, A. A. (2020). Leaf pigments and chlorophyll fluorescence patterns in leaves of mango as affected by low temperature degrees under field and laboratory conditions. *Horticulture International Journal*, 4(1), 16–26. <https://doi.org/10.15406/hij.2020.04.00151>
- Ghoreishi, M., Hossini, Y., & Maftoon, M. (2012). Simple models for predicting leaf area of mango (*Mangifera indica* L.). *Journal of Biology and Earth Sciences*, 2(2), B45–B53.
- Gomez-Rubio, V. (2017). Book Review: ggplot2 – Elegant Graphics for Data Analysis (2nd ed.) by Hadley Wickham. *Journal of Statistical Software*, 77(2), 1–3. <https://doi.org/10.18637/jss.v077.b02>
- Igbari, A. D., Nodza, G. I., Adeusi, A. D., & Ogundipe, O. T. (2019). Morphological characterization of mango (*Mangifera indica* L.) cultivars from south-west Nigeria. *Ife Journal of Science*, 21(1), 155–167. <https://doi.org/10.4314/ijfs.v21i1.13>
- Indian, G., Sankaranarayanan, R., Murugesan, S., & Rajangam, J. (2022). Studies on genotypic and phenotypic variability in mango (*Mangifera indica* L.). *Journal of Agriculture and Ecology*, 13, 121–130. <https://doi.org/10.53911/JAE.2022.13112>
- Jadhav, S. D., Shinde, V. N., & Kakade, V. (2022). Characterization of leaves and fruits of mango (*Mangifera indica* L.). *The Pharma Innovation Journal*, 11(4), 411–416.
- Jutamanee, K., & Onnom, S. (2016). Improving photosynthetic performance and some fruit quality traits in mango trees by shading. *Photosynthetica*, 54(4), 542–550. <https://doi.org/10.1007/s11099-016-0210-1>
- Kaur, H., Sidhu, G., Mittal, A., & Chhuneja, P. (2024). Deciphering the molecular dynamics of mango flowering and malformation: Insights from RNA-Seq analysis [Poster]. *Mango Symposium*. <https://www.researchgate.net/publication/378766011>
- Kumar, R., Raj, A., Prasad, M., Sahay, S., & Kushwaha, C. (2018). Assessing the flowering and fruiting behaviour in some important cultivars of mango (*Mangifera indica* L.). *British Journal of Applied Science & Technology*, 25(5), 1–8. <https://doi.org/10.9734/CJAST/2018/45855>
- Lichtenthaler, H. K., & Wellburn, A. R. (1981). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11(5), 591–592.
- Lucena, C. C. de, Siqueira, D. L. de, Martinez, H. E. P., & Cecon, P. R. (2012). Salt stress changes chlorophyll fluorescence in mango. *Revista Brasileira de Fruticultura*, 34(4), 1245–1255. <https://doi.org/10.1590/S0100-29452012000400017>
- Majumder, D. A. N., Hassan, L., Rahim, M. A., & Kabir, M. A. (2012). Correlation

- and path coefficient analysis of mango (*Mangifera indica* L.). *Bangladesh Journal of Agricultural Research*, 37(3), 493–503.
- Makowski, D., Ben-Shachar, M., Patil, I., & Ludecke, D. (2020). Methods and Algorithms for Correlation Analysis in R. *Journal of Open-Source Software*, 5:2306. <https://doi.org/10.21105/joss.02306>
- Niaz, N., Gulzar, S., Kazmi, J. H., Aleem, S., Pham, M. P., Mierzwa-Hersztek, M., & Mushtaq, Z. N. (2024). Assessment of chlorophyll content in leaves of crops and orchards based on SPAD, multispectral, and hyperspectral techniques. *Ecological Questions*, 35(2), 161–174. <https://doi.org/10.12775/EQ.2024.025>
- Pike, T. (2022). qboxplot: Quantile-Based Boxplot (R package version 0.2). <https://CRAN.R-project.org/package=qboxplot>
- Plavcova, L., Mészáros, M., Silhan, K., & Jupa, R. (2022). Relationships between trunk radial growth and fruit yield in apple and pear trees on size-controlling rootstocks. *Annals of Botany*, 130(4), 477–489. <https://doi.org/10.1093/aob/mcac089>
- Rani, R., Jaiswal, U. S., Kumar, A., & Bhamini, K. (2018). The total leaf chlorophyll content and malformation relationship in mango (*Mangifera indica* L.) plants growing under stressful condition. *Multilogic in Science*, VIII (Special C), 126–130.
- Reddy, Y. T. N., & Singh, G. (1991). Further studies on the relationship between leaf number and area and fruit development in mango (*Mangifera indica* L.). *Journal of Horticultural Science*, 66(4), 471–478. <https://doi.org/10.1080/00221589.1991.11516176>
- Rich, P. M., Chen, J., Sulatycki, S. J., Vashisht, R., & Wachspress, W. S. (1995). Calculation of leaf area index and other canopy indices from gap fraction: A manual for the LAICALC software. Kansas Applied Remote Sensing Program Open File Report.
- Rymbai, H., Laxman, R. H., Dinesh, M. R., Sunoj, V. S. J., Ravishankar, K. V., & Jha, A. K. (2014). Diversity in leaf morphology and physiological characteristics among mango (*Mangifera indica* L.) cultivars popular in different agro-climatic regions of India. *Scientia Horticulturae*, 176, 189–193. <https://doi.org/10.1016/j.scienta.2014.06.030>
- Samant, A., Haldankar, P. M., Bhuwad, A., Warang, O. S., & Patil, A. P. (2020). Relationship between plant canopy volume, leaf area index and yield in mango (*Mangifera indica* L.) cv. Alphonso. *Journal of Applied and Natural Science*, 12(4), 673–677.
- Schaffer, B., & Gaye, G. O. (1989). Gas exchange, chlorophyll and nitrogen content of mango leaves as influenced by light environment. *HortScience*, 24(3), 507–509.
- Singh, V. K., Tiwari, A. K. M., Singh, D. K., & Pathak, S. M. (2007). Effect of leaf number and area on the fruit growth of regular and biennial bearing mango (*Mangifera indica* L.) grown under North Indian conditions. *International Journal of Fruit Science*, 6(4), 77–91. https://doi.org/10.1300/J492v06n04_08
- Sinha, A., Mir, H., Rani, R., & Prasad, B. D. (2018). Studies on floral biology and leaf characteristics of mango hybrids and their parents. *British Journal of Applied Science & Technology*, 25(3), 1–9. <https://doi.org/10.9734/CJAST/2018/45986>
- Sridhar, D., Ghosh, B., Das, A., & Pramanik, K. (2022). Morphological characterization of Mango (*Mangifera indica* L.) Cultivars. *Biological Forum – An International Journal*, 14(1): 1676–1682.
- Urban, L., Le Roux, X., Sinoquet, H., Jaffuel, S., & Jannoyer, M. (2003). A biochemical model of photosynthesis for mango leaves: Evidence for the effect of fruit on photosynthetic capacity of nearby leaves. *Tree Physiology*, 23(5), 289–300. <https://doi.org/10.1093/treephys/23.5.289>