International Journal of Sciences, Engineering & Environmental

Technology (IJOSEET), 16(16): 19-27, 2024
ISSN 0749-9650

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Assessment of Leaf Area Metrics in *Tectona grandis*: Integrating Manual and Photographic Methods with Seasonal and Environmental Considerations

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Abstract- *This study evaluates leaf area metrics in Tectona grandis, integrating manual and photographic measurements with considerations for seasonal and environmental influences. Conducted across two forest reserves in Nigeria, the research investigated differences between manually and photographically derived leaf metrics, explored the effects of seasonality and location on leaf metrics, and develops predictive models for leaf area based on linear measurements. Leaf samples were collected during both wet and dry seasons, with manual measurements for length and breadth compared to image-derived metrics processed through ImageJ software. The findings revealed a strong positive correlation between the two measurement techniques, though biases were observed, particularly in leaf area, where manual estimates were significantly higher. Seasonal variations were evident, with dry season measurements showing consistently larger leaf dimensions across sites, indicating possible adaptive responses of T. grandis to water availability. Multiple regression models based on leaf dimensions were developed, with the optimal model achieving high accuracy (Adj-R^2 = 0.97). This work demonstrates the reliability of non-destructive, image-based leaf area estimation and underscored the impact of seasonal changes on leaf metrics, providing a foundation for further ecological and physiological studies of T. grandis.*

Keywords: *Allometric equations, Leaf metrics, Manual measurement, Photographic measurement, Seasonal variation, Tectona grandis,*

1.0 Introduction

Leaf area is a fundamental parameter in plant biology, directly influencing a plant's capacity to capture sunlight and perform photosynthesis effectively, thus impacting its growth, productivity, and overall health (Ukonmaanaho *et al.*, 2016). Measuring leaf area and its index provides insights into the ecological functions of plants, including their roles in transpiration, carbon sequestration, and energy distribution within ecosystems (Neinavaz *et al.*, 2016). Given the importance of leaf area as a metric, numerous methodologies have been developed for its measurement, ranging from direct approaches like laser planimetry and image analysis (Granier *et al.*, 2002) to regression models that leverage linear measurements for estimation (Ogoke *et al*., 2004).

Allometric models have proven valuable for estimating leaf area based on leaf dimensions, such as length and breadth, offering a non-destructive, efficient technique for field studies (Tondjo *et al.*, 2015). Studies on species like *T. grandis* have utilized these models to link leaf area with biomass and structural growth, highlighting their applicability across different environmental and ecological contexts (Vyas *et al.*, 2010). This paper builds upon these foundational approaches to leaf area assessment. The objectives of this study includes:

1. To assess the difference between manually and photographically measured leaf metrics of *T. grandis.*
2. To determine the effect of seasonality and location on the leaf metrics of *T. grandis*.
3. To model leaf area of *T. grandis* using linear measurements as independent variables.

2.0 Methods

2.1 Map of Study Sites:

Fig 2.1 illustrates the geographical locations of Onigambari and Ugbolu Forest Reserves, which serve as the primary study sites for this research. Onigambari Forest Reserve is situated in Idi-Ayunre, Ibadan, within Oyo State, while Ugbolu Forest Reserve is located in Ugbolu, Asaba, in Delta State.

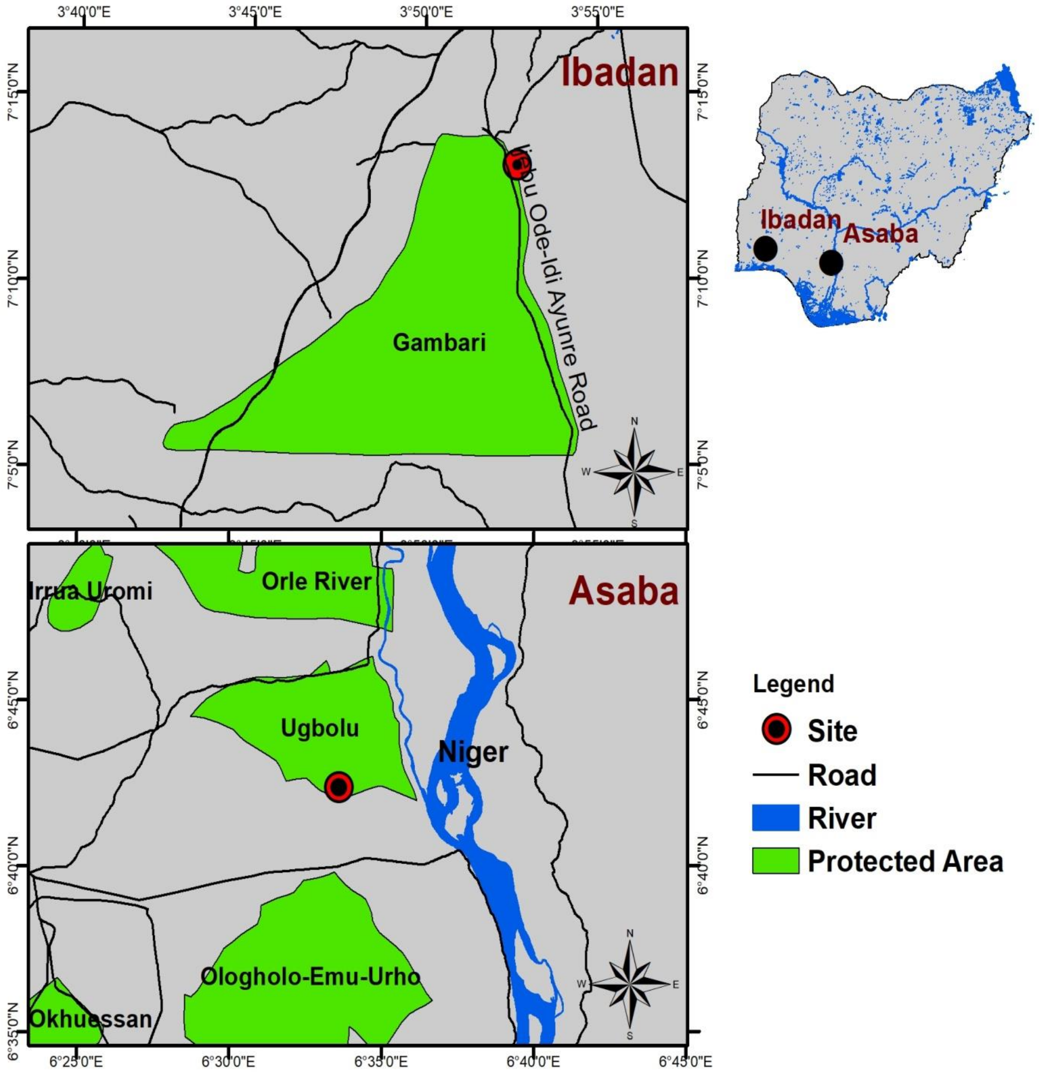

Fig 2.1 Map of Onigambari and Ugbolu Forest Reserves (in set: Map of Nigeria)

2.2 Leaf Metrics (Length, Breadth and Area)

During wet and dry seasons, a selection of mature and fully developed leaves was randomly collected from each plantation in January and May, respectively. Care was taken to include leaves of various sizes, ranging from the smallest to the largest (Table 2.1). Each individual leaf was labelled and measured using a graduated tape rule. The length of the leaf was recorded by measuring from the base of the petiole along the midrib to the apex of the lamina, while breadth measurement was done at the widest point perpendicular to the midrib (Plate 2.1). To capture detailed images, the leaves were flattened on a white cardboard and photographed in a vertical position using a digital single lens reflex camera equipped with a 50 mm lens. The camera settings were adjusted to automatic mode, and for accurate scaling, a reference object of a known size was positioned next to each leaf in the photographs. Subsequently, the acquired images were processed and analysed with Image J version 2 (Rueden *et al.*, 2017) (Plate 2.2). In Image J, the tools were used to manually measure and extract the length, width, and area of each leaf from the scaled images and recorded in a spreadsheet, alongside the corresponding manual measurements.

Manual estimates of leaf area were computed by multiplying manually measured length and breadth of individual leaves. Data were pooled across sites and seasons and a paired t-test was conducted to evaluate the variations between estimates obtained manually and those derived from images of leaf length, breadth and area. The Pearson correlation coefficient was used to assess the relationship between the two methods. The bias between the two methods for each leaf measurement was calculated using the following approach:

$$B = 100\left[\frac{1}{n}\sum_{i=1}^{n}\left(\frac{M_{(i)} - I_{(i)}}{I_{(i)}}\right)\right]$$

Where I(i) and M(i) represent the measurements obtained from images and manual methods, respectively, for leaf i, and n denotes the total count of leaves

Assessments of seasonal and site-dependent effects on leaf metrics were done through a two way Analysis of Variance, on leaf length, breadth and area. Using image derived leaf metrics, linear regression models were explored in order to identify the best predictor of leaf area determined by measuring both the length and breadth of the leaf. Model goodness-of-fit was assessed using the Adjusted coefficient of determination (Adj-R^2). The predictive ability of the best model was tested using manual linear measurements. All analyses were done in R version 3.6.0.

Table 2.1: Number of Leaf samples of *Tectona grandis* collected From Plantations at Ibadan and Asaba, Nigeria.

Location	Dry season number of leaves	Rainy season number of leaves	Total
Ibadan	72	110	182
Asaba	97	130	227
Total	169	240	409

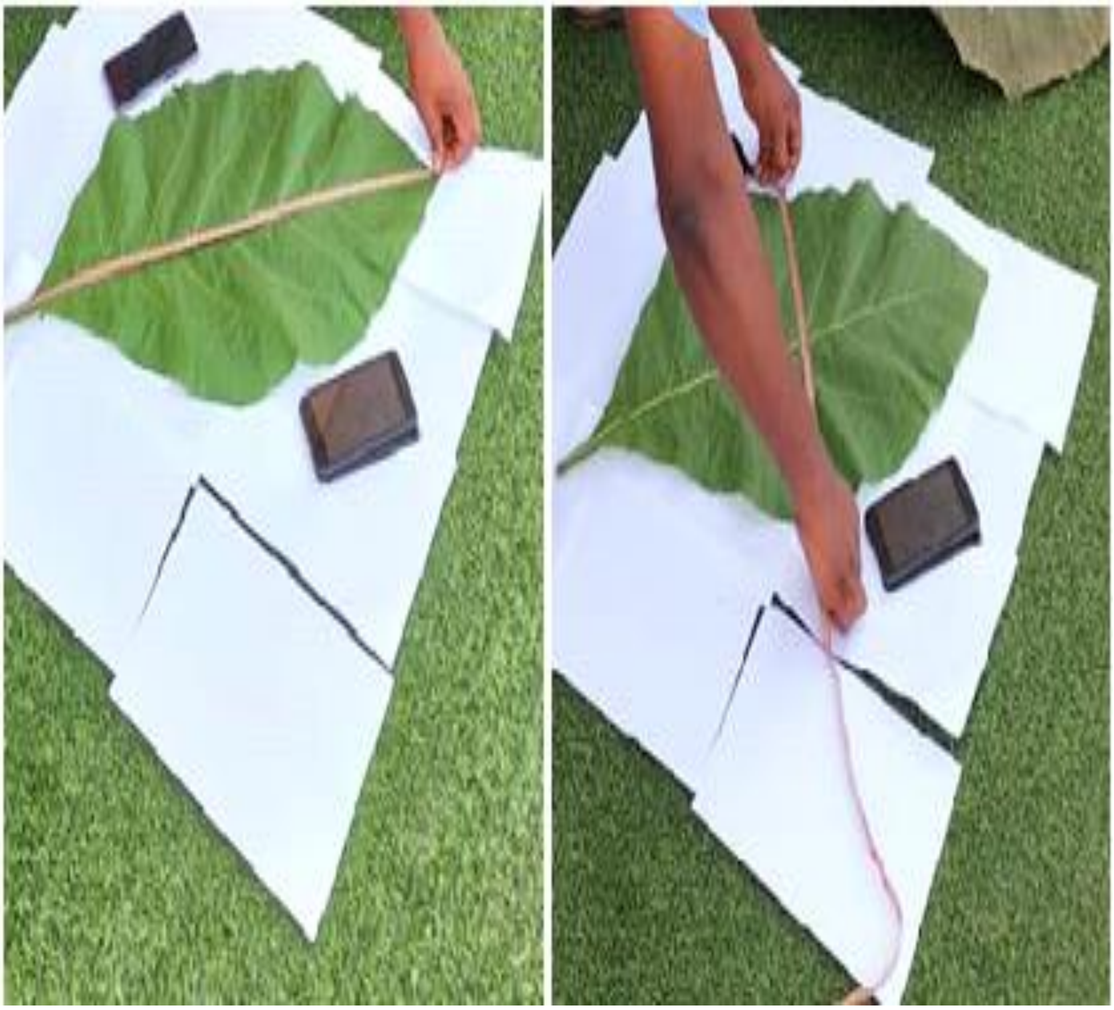

Plate 2.1: Manual measurement of length and breadth of *Tectona grandis* leaves

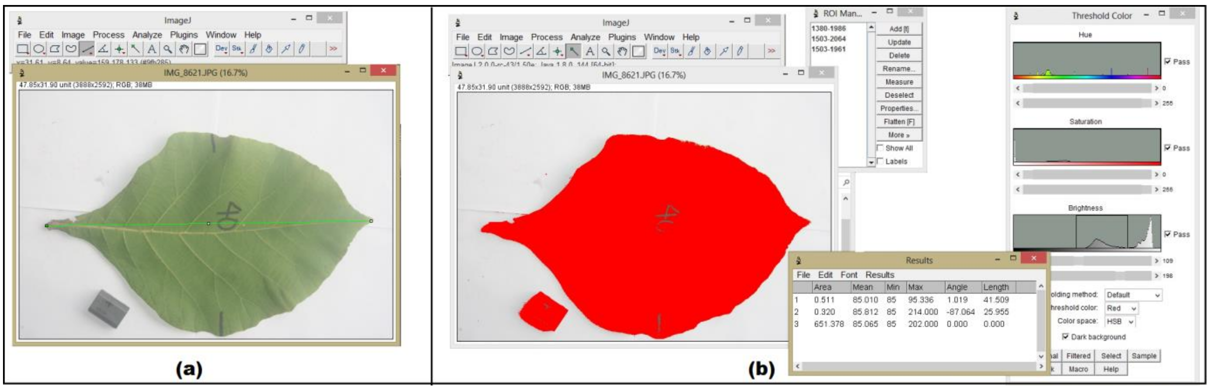

Plate 2.2: The graphical user interface of imageJ showing an image prior to processing (a) and after extraction of metrics (b).

3.0 Results

There were no significant differences in all leaf metrics. Though manual estimates were numerically higher than image-based estimates, the biases for leaf length and breadth were very low (< 0.5 %) but very high for leaf area (Table 3.1). Correlation analysis showed a very strong and positive relationship between manual and image based methods (Figure 3.1).

Table 3.1: Means (±SEM) of leaf metrics of *T. grandis* measured manually and photographically (n = 409)

	Manual	Image	*p*-value	Bias (%)
Leaf length (cm)	55.50 ± 0.52	55.40 ± 0.52	0.033	0.27
Leaf breadth (cm)	33.69 ± 0.38	33.56 ± 0.38	0.001	0.47
Leaf area (cm^2)	1937.01 ± 37.79	1178.97 ± 24.19	<0.001	69.34

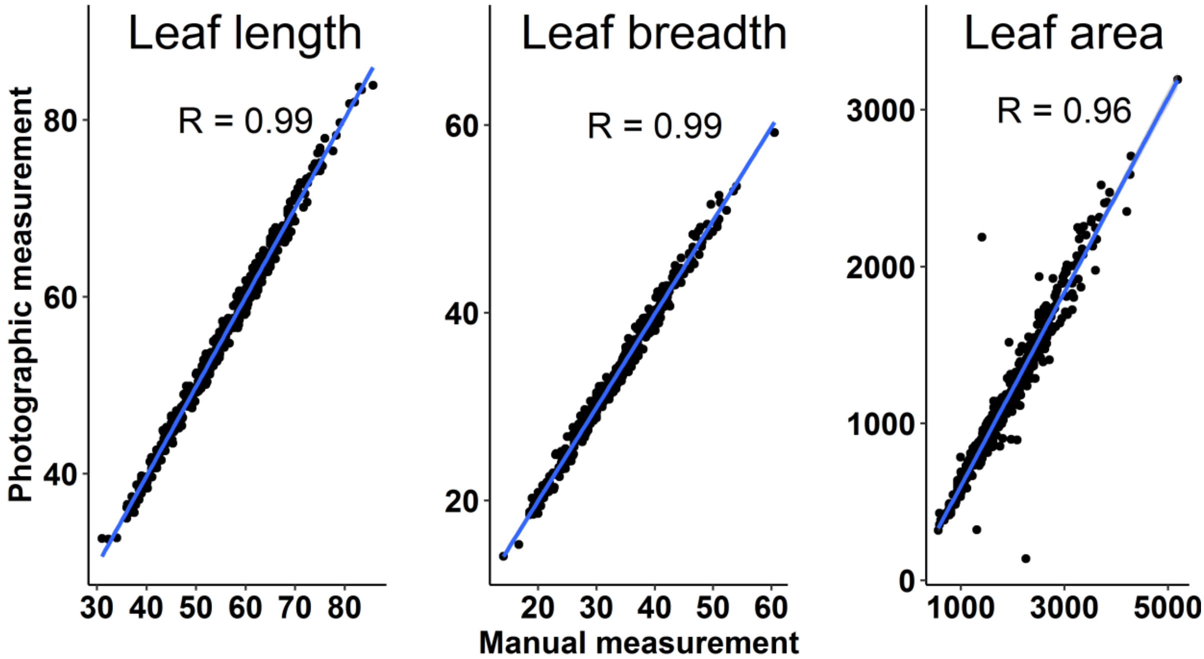

Figure 3.1: Relationship between manually estimated and image derived leaf metrics of *T. grandis* from two plantations in Nigeria (n = 409).

All leaf metrics were significantly higher during the dry season for both sites (Table 3.2). There was a significant difference in leaf length and breadth between both sites, with Ibadan showing higher values of these metrics. However, leaf area did not differ between sites. The interaction between site and season was significant for all metrics.

Table 3.2: Two-way ANOVA summary results of leaf metrics of *T. grandis* during the dry and rainy season in two plantations in Nigeria.

	Summary ANOVA			Group means ±SE			
	Season	Site	Season×Site	Ibadan		Asaba	
				Dry	Rainy	Dry	Rainy
Length	***	*	***	65.16±0.89	50.75±0.97	56.54±1.16	53.08±0.69
Breadth	***	*	***	38.38±0.70	28.92±0.70	36.61±0.79	32.53±0.53
Area	***	n.s.	**	1492.79±50.44	934.03±40.37	1353.74±57.34	1082.00±31.49

n.s. not significant; *$p < 0.05$; ** $p < 0.01$; ***$p < 0.001$

3.1 Leaf area models for *Tectona grandis* from plantations

Allometric equation for leaf area estimation (Table 3.3) indicated that in Model 1, the independent variable was leaf length. This model produced the lowest adjusted R^2, the highest AIC and BIC values. Model 2 was a quadratic allometric equation based on length. This model performed better than the Model 1 as it yielded relatively better Adjusted R^2, AIC and BIC values. Model 3 and Model 4 were simple linear and quadratic equations respectively based on leaf breadth. These models generally performed better than the two previous models with Adjusted R^2 which is higher than the previous two. Model 5, a multiple linear model based on length and breadth showed a better Adjusted R^2, AIC and BIC values, than all the previous models. Model 6 was the allometric equation that best predicated the leaf area of *T. grandis* as it has the highest value of Adjusted R^2, which is closest to 1, as well as the lowest values of AIC and BIC. A strong correlation was found between the predicted leaf area of *Tectona grandis* using linear measurements and the optimal model for leaf area estimation, represented by the equation ($A = 0.62lb - 15.55$) (Figure 3.2).

Table 3.3: Equations and statistical estimators derived from the analysis of correlation between image derived linear metrics and their combinations and leaf area of *T. grandis* from two plantations in Nigeria.

Model	Variable	Equation	Adj-R^2	AIC	BIC
1	L	$A = 41.52l - 1121.32$	0.81	5554.82	5566.86
2	l^2	$A = 0.32l^2 + 5.29l - 137.8$	0.82	5538.71	5554.76
3	B	$A = 59.68b - 823.72$	0.88	5373.19	5385.23
4	b^2	$A = 0.78b^2 + 6.12b + 52.63$	0.89	5317.80	5333.86
5	l +b	$A = 17.51l + 39.31b - 1110.08$	0.92	5205.77	5221.83
6	l*b	$A = 0.62lb - 15.55$	0.97	4711.70	4723.72

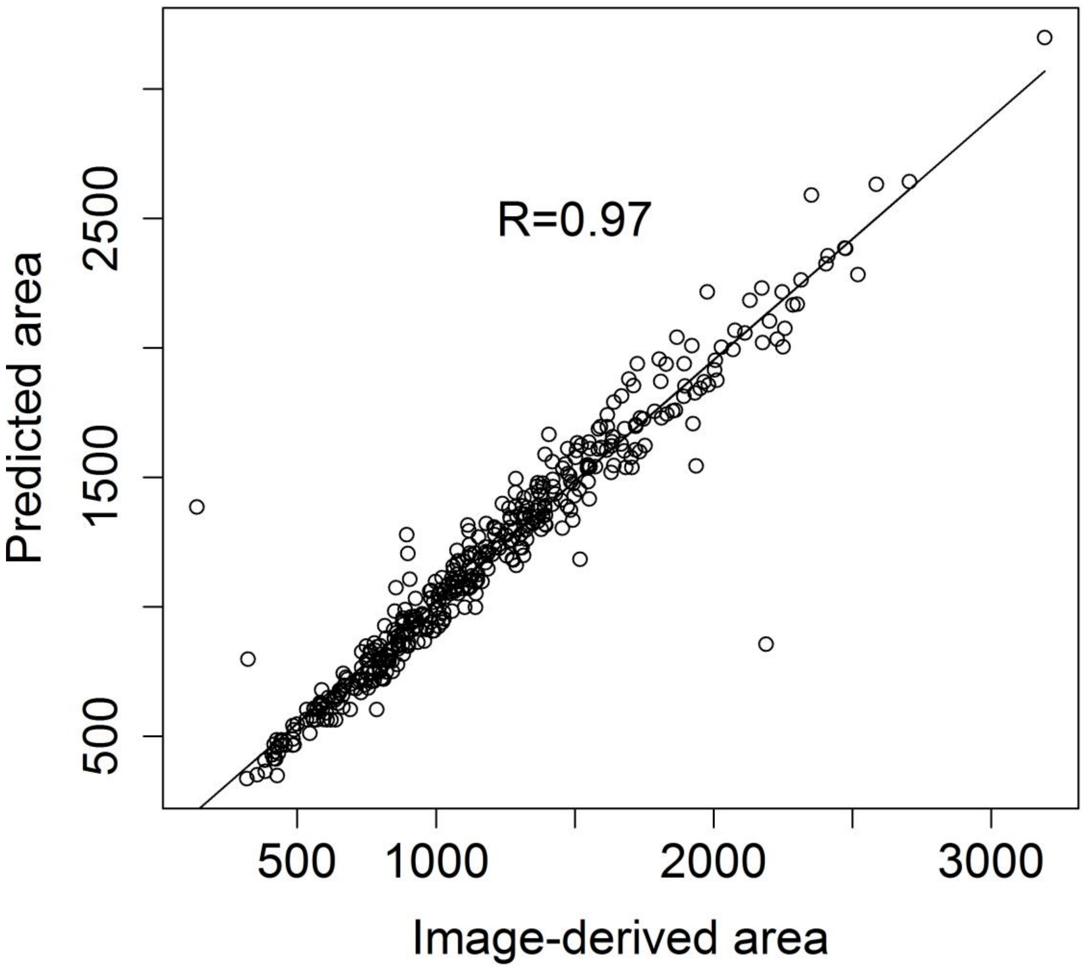

Figure 3.2: Predicted leaf area of *T. grandis* based on linear measurements and the best model for leaf area ($A = 0.62lb - 15.55$).

4.0 Discussion and Conclusions

Results by Tondjo *et al.* (2015), where they developed a linear equation A = 0.60 × L × Wi, to estimate leaf area from the product of length and width in *T. grandis* in Togo, had a coefficient similar to my study's regression model. Their model, closely aligns with our best leaf area regression model, A = 0.62lb - 15.55, indicating a strong relationship between leaf area and leaf dimensions across different geographical locations. This similarity suggests that the relationship between leaf length, breadth, and area in *T. grandis* is consistent across different environments, supporting the use of linear models for leaf area estimation.

Furthermore, study by Vyas *et al*. (2010) in Gujarat, India, provided additional support for the validity of my findings. They used various methods, including destructive sampling and the photo-grid method, to calculate the Leaf Area Index (LAI) of *Tectona grandis*. Their results demonstrated that the LAI values obtained from their developed allometric equation were comparable to those from direct measurement methods, with minimal error. This supports the idea that image-derived methods, like those used inour study, can provide accurate estimates of leaf metrics, particularly when linear relationships are applied. Although this study did not specifically focus on LAI, the consistent accuracy across different methods in estimating leaf area further validates the reliability of our findings.

In addition, our results agree with that of Leroy *et al.* (2007). They also provided relevant information into the use of non-destructive methods for estimating leaf area. Leroy *et al.* (2007) concluded that digital photography and linear measurements could effectively estimate leaf area in agroforestry systems, which aligns with the high accuracy observed in my image-derived leaf length and breadth measurements.

There were significant seasonal variations in leaf metrics at both sites. During the dry season, all leaf metrics—length, breadth, and area—were notably higher compared to the rainy season. This observation aligns with the findings of Wagner *et al.* (2014), who stated that climate conditions can influence the length of the growing season, thereby impacting tree growth in subsequent seasons.

In conclusion, our results indicated that image-derived leaf metrics of *T. grandis* did not differ significantly from their respective manual estimates and that the image-derived leaf metrics were more accurate compared to manual measurements. The observed increase in *T. grandis* leaf metrics during the dry season compared to the rainy season may be attributed to the plant's adaptive mechanisms for water conservation and maximized photosynthetic efficiency under stress conditions. During drier periods, many tree species, including *T. grandis*, often exhibit thicker, more robust leaves with larger surface areas as part of a strategy to enhance water-use efficiency and resist desiccation. This phenomenon is supported by studies by Carmo *et al.* (2022), which indicated that *T. grandis* adjusts its growth patterns to optimize leaf surface and maintain productivity in response to seasonal moisture availability. Similarly, Vyas *et al.* (2010) found that seasonal leaf morphology changes are a critical adaptive response, helping species in semi-arid environments maintain high photosynthetic rates and biomass accumulation despite fluctuating water availability. These findings suggest that leaf metric variations in *T. grandis* are an ecological adaptation to balance growth and water conservation across seasons.

The evaluation of models for predicting leaf area in *T. grandis* demonstrated the superiority of quadratic allometric equations utilising leaf length and breadth as predictors. These models outperformed the simpler linear allometric equations and highlighted the significance of considering both leaf dimensions. The findings aligned with previous research by Tondjo *et al*, 2015, reinforcing the importance of the product of length and breadth in accurately estimating leaf area.

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