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Morphological traits and non-destructive leaf area estimation of *Moringa oleifera* under different stem cutting lengths

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Abstract. *Gustiar F, Ria RP, Krhisma NA. 2026. Morphological traits and non-destructive leaf area estimation of* Moringa oleifera *under different stem cutting lengths. Biodiversitas 27 (5): d270523. https://doi.org/10.13057/biodiv/d270523. Moringa oleifera* is widely cultivated for its highly nutritious leaves, making efficient propagation and accurate growth evaluation essential for improving leaf productivity. This study aimed to evaluate the effects of different stem cutting lengths on the morphological traits and early growth of *M. oleifera* and to develop a non-destructive method for estimating leaf area. The experiment was conducted in a greenhouse using a randomized complete block design with five stem cutting lengths (20, 30, 40, 50, and 60 cm). Leaf area estimation models were developed using measurements of leaf length and width of fully expanded compound leaves using regression analysis. Among the evaluated models, the linear regression based on leaf Length × Width (L × W) produced the highest predictive performance (R^2 = 0.9636) within the calibration dataset used in this study. Stem cutting length was associated with variation in early growth responses. Cuttings of approximately 40 cm generally showed more favorable early-growth characteristics, including higher survival percentage, larger leaf area, and greater dry biomass accumulation compared with several other treatments. Because survival data were derived from a limited number of plants per treatment, survival responses are interpreted descriptively. Differences in fresh biomass variables were not statistically significant, although variation was observed in leaf and root dry weight. These findings suggest that moderate cutting length may support balanced early establishment of *M. oleifera* under greenhouse conditions. The developed leaf area model provides a practical tool for repeated growth monitoring without destructive sampling. However, because the model was not validated using an independent dataset, further testing under field conditions and across broader environmental settings is still required before wider application.

Keywords: *Kelor*, non-destructive, perennial vegetable, propagation, regression

INTRODUCTION

Moringa oleifera Lam. is a fast-growing perennial leafy vegetable species belonging to the Moringaceae family, native to the Indian subcontinent and now widely distributed in tropical and subtropical regions (Anzano et al. 2021). The species is well adapted to warm climates with high solar radiation and can tolerate relatively poor soil fertility and seasonal drought. This plant is also used to support food security in climate-resilient farming practices (Lillo et al. 2025). Morphologically, *M. oleifera* is characterized by soft woody stems and tripinnate compound leaves composed of numerous leaflets (Singh et al. 2020). The species is widely recognized as the “tree of life” because of its exceptional nutritional and medicinal value. The leaves contain high concentrations of vitamins A, C, and E, essential amino acids, minerals such as calcium and iron, and bioactive compounds including phenolics and flavonoids with strong antioxidant properties (Anzano et al. 2021; Islam et al. 2021). As demand for *Moringa*-based products continues to increase, the availability of uniform and high-quality planting material has become increasingly important for sustainable leaf production systems (Kudzinawo et al. 2022).

Vegetative propagation through stem cuttings is commonly used in *Moringa* cultivation because it maintains genetic uniformity and enables rapid establishment compared with seed propagation. However, the success of this method depends on several factors, including cutting length, stem diameter, number of nodes, and carbohydrate reserves stored in the cutting tissues (Pamungkas et al. 2021; Gustiar et al. 2024). Previous studies have shown that cutting size influences early growth performance. For instance, longer cuttings (50-75 cm) have been reported to enhance seedling growth (Santoso and Arya Parwata 2020; Muniandi et al. 2024), while recent findings indicate that variation in cutting length affects rooting capacity, shoot elongation, and biomass accumulation (Tailor et al. 2023; Mafrica et al. 2025). Nevertheless, the optimal cutting length for balancing shoot growth, rooting performance, and canopy development in *Moringa* remains insufficiently understood.

Leaf area is an important indicator of plant growth because it determines the photosynthetic surface available for light interception and carbon assimilation (Sukhova et al. 2022). In *Moringa*, leaf development is particularly important because the leaves constitute the main harvested and economically valuable component (Muniandi et al. 2024). Conventional leaf area measurement, however, generally requires destructive sampling, which may interfere with repeated observations during early growth stages. Therefore, non-destructive approaches are needed to allow continuous growth monitoring without damaging plant structure.

Non-destructive leaf area estimation methods using morphometric relationships between leaf dimensions and actual leaf area have been widely applied in various crop species and provide reliable predictions (Lakitan et al. 2022; Gustiar et al. 2023b). However, most of these studies have focused on simple-leaf species. In compound-leaf species such as *M. oleifera*, leaf area estimation is more complex because leaflet number, leaflet arrangement, leaflet overlap, and expansion pattern can vary substantially among leaves (Tsaniklidis et al. 2025). As a result, simple linear measurements often cannot accurately represent the total photosynthetic surface of the compound leaf. To date, studies integrating vegetative propagation responses with calibrated non-destructive leaf area estimation in compound-leaf *Moringa* are still very limited. Most previous studies have evaluated propagation success and leaf growth separately, making it difficult to develop integrated nursery management strategies that combine propagation efficiency with practical growth assessment tools. Unlike previous studies, the present study simultaneously evaluated stem cutting performance and developed a non-destructive leaf area model within the same experimental framework. This approach enables rapid and repeated monitoring of seedling growth without destructive sampling.

Therefore, this study was conducted to integrate stem cutting propagation evaluation with non-destructive leaf area modeling in *M. oleifera*. Specifically, the study aimed to evaluate the effects of different stem cutting lengths on early vegetative growth and to develop a practical non-destructive model for estimating leaf area during early plant establishment. Although previous studies have separately examined vegetative propagation or leaf growth characteristics in *Moringa*, integrated studies combining propagation performance with calibrated non-destructive leaf area estimation are still very limited, particularly for compound-leaf species. Thus, this study contributes an integrated approach that supports both propagation management and rapid growth monitoring in *Moringa* nursery systems. It was hypothesized that moderate cutting length would provide more balanced early growth performance, while combined leaf dimensions, particularly leaf length × width, would provide more accurate leaf area estimation than single morphometric predictors.

MATERIALS AND METHODS

Site and experimental design

The experiment was conducted in a greenhouse at the Department of Agronomy, Faculty of Agriculture, Universitas Sriwijaya, Indralaya, Ogan Ilir District, South Sumatra, Indonesia, from June to September 2025. The study was arranged in a Randomized Complete Block Design (RCBD) with one factor, namely stem cutting length of *M. oleifera*. Five cutting length treatments were evaluated: P1: 20 cm, P2: 30 cm, P3: 40 cm, P4: 50 cm, and P5: 60 cm. Each treatment was replicated three times, with four plants per experimental unit, resulting in a total of 60 plants (5 treatments × 3 replications × 4 plants). Blocking was used to account for environmental gradients within the greenhouse, especially possible variation in light intensity and temperature along bench position. Each block represented a relatively homogeneous microenvironment, and all treatments were randomly assigned within each block. *M. oleifera* is a tree species characterized by compound leaves composed of numerous leaflets (Figure 1). Stem cuttings used as planting materials were obtained from the experimental field of Universitas Sriwijaya. Cuttings were collected from healthy, disease-free mother plants aged approximately 3-4 years, with uniform physiological condition. The cuttings were taken from the middle portion of the stem to ensure consistency in physiological status. Stems with relatively similar diameters were selected to minimize variation associated with differences in carbohydrate reserves, with an initial diameter ranging from 18-22 mm. The stems were cut according to the respective treatment lengths and planted in polybags containing a mixture of top soil and well-decomposed cattle manure at a ratio of 3:1 (v/v). The medium was thoroughly mixed to ensure homogeneity and adequate drainage. Each polybag contained one cutting, which was planted vertically with approximately one-third of its length inserted into the growing medium, ensuring consistent node exposure above the soil surface. All experimental units were arranged in the greenhouse according to the experimental design. During the experimental period, plants were grown under general greenhouse conditions with natural light and no shading. Irrigation was applied daily to maintain adequate soil moisture without waterlogging. No additional fertilizers were applied during the early growth stage, and all treatments received uniform maintenance throughout the experiment.

Growth observation and leaf area estimation

Plant growth observations were conducted during the early growth stage and included plant height, number of leaves, and leaf area. Plant height was measured from the surface of the growing medium to the highest growing point of the plant. The number of leaves was recorded by counting fully expanded leaves. Leaf area was estimated using a non-destructive regression approach based on the relationship between leaf Length (L), leaf Width (W), number of leaflets, and actual Leaf Area (LA) (Figure 2). Regression models were developed to obtain predictive equations, allowing subsequent measurements to be conducted without damaging the leaves. For model development, a total of 150 fully expanded compound leaves were sampled. Leaves were collected across treatments and developmental sizes to capture variation in leaf morphology. The morphological unit used in the regression analysis was the entire compound leaf, while leaflet number was included as an explanatory variable to account for variation in leaflet density and arrangement affecting total leaf area. Leaf length and width were measured manually using a ruler, with one measurement point per leaf. Leaf length was measured from the base to the tip along the main rachis, while leaf width was measured at the widest part of the leaf.

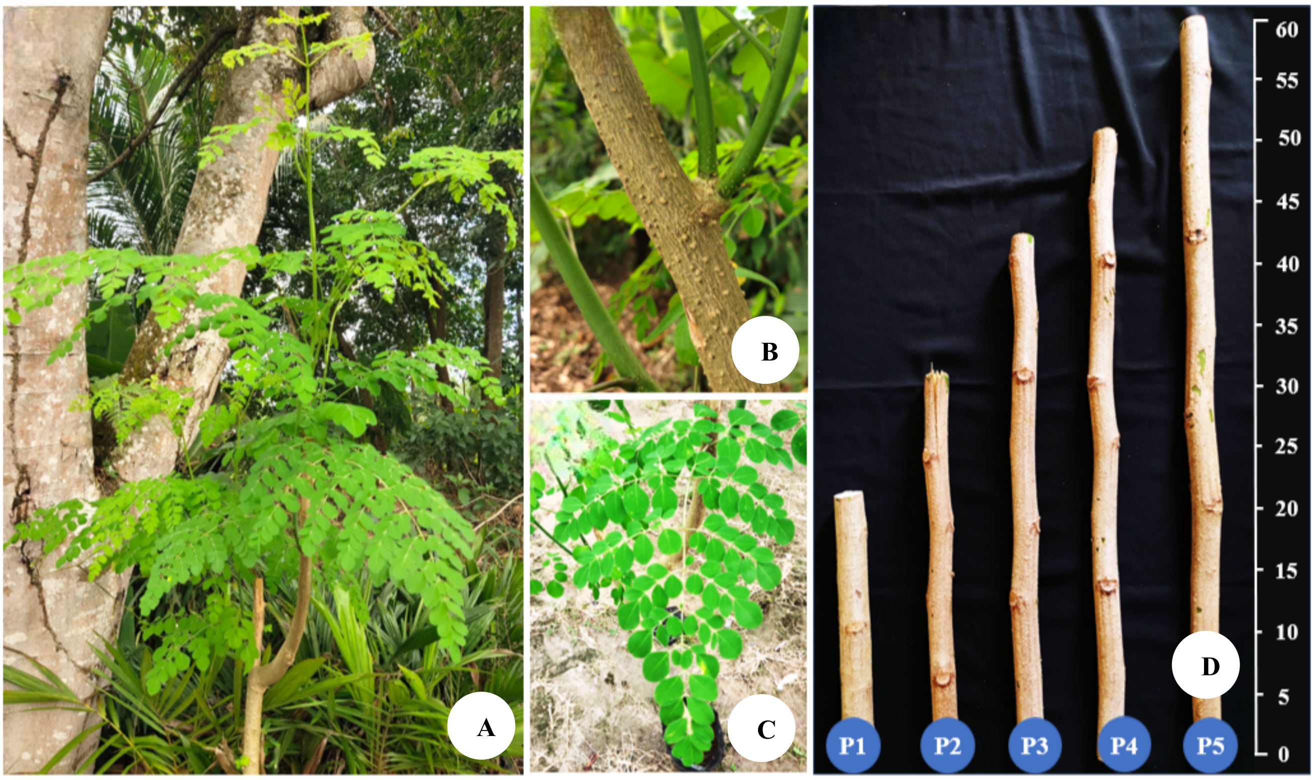

Figure 1. Morphology of *Moringa oleifera* and stem cutting materials used in the experiment: A. Plant morphology, B. Stem and node structure, C. Compound leaves with leaflets, and D. Stem cuttings with different lengths (20-60 cm)

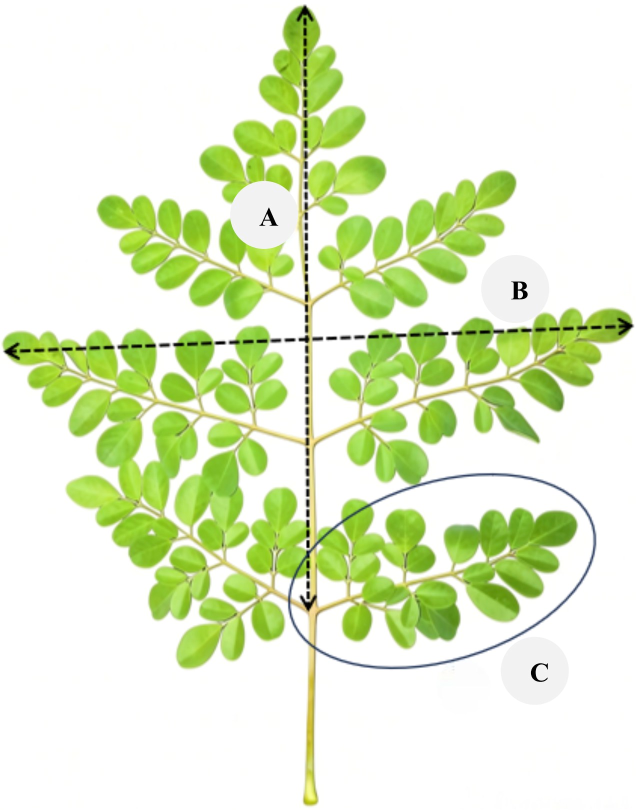

Figure 2. The compound leaf structure of *Moringa oleifera* and the measurement method of: A. Leaf length, B. Leaf width, C. Leaflets

To obtain actual leaf area for model calibration, fully expanded leaves were photographed using a digital camera against a black background to improve image segmentation. Images were captured at a fixed distance of 50 cm under ambient greenhouse light conditions without artificial control. Leaf area and canopy area were determined using the Easy Leaf Area application (Gustiar et al. 2024), which employs a color thresholding approach with a standardized reference object. In this study, a red reference square (2 × 2 cm) was included in each image and used for area calibration. Although imaging conditions were standardized in terms of distance, background, and calibration object, variation in ambient light intensity within the greenhouse may have introduced minor bias during image segmentation and area detection. However, all images were processed using identical threshold settings to minimize analytical inconsistency among samples.

Canopy area was measured from digital images of the plant canopy and expressed as projected canopy surface area. In this study, canopy area represents the projected two-dimensional area of the shoot canopy, whereas leaf area refers to the estimated total area of the compound leaves based on the developed regression model. Thus, canopy area and leaf area describe different aspects of plant development and should not be interpreted as equivalent variables.

As a supporting physiological parameter, leaf chlorophyll content was measured using a SPAD meter (Konica Minolta SPAD-502). SPAD readings were taken from three fully expanded leaves per plant, with one measurement point per leaf at the middle leaflet area. This sampling intensity was selected to provide representative chlorophyll measurements while minimizing destructive disturbance and excessive handling during repeated observations in young plants. The mean value per plant was used for statistical analysis.

Statistical analysis

For growth, root, and biomass variables, the experimental unit was the plot mean, corresponding to the mean value of the four plants within each experimental unit, in accordance with the RCBD structure. Data were analyzed to evaluate the effect of stem cutting length on the early growth of *M. oleifera*. Growth variables were subjected to Analysis of Variance (ANOVA) using a Randomized Complete Block Design (RCBD), with cutting length as the treatment factor and block as the replication factor. Prior to ANOVA, assumptions of normality and homogeneity of variance were examined. When significant treatment effects were detected, mean comparisons were performed using Tukey's HSD test at the 5% significance level.

Survival percentage was calculated as the proportion of surviving plants per experimental unit. Because of the limited number of plants per unit and because proportional survival data were not reanalyzed using a generalized linear model in the present manuscript revision, survival is presented descriptively and interpreted cautiously. Weekly observations of shoot number, shoot length, branch number, and leaf number from 1 to 11 Weeks After Planting (WAP) are presented as descriptive growth trajectories. Because repeated-measures or longitudinal statistical analysis was not performed in the present study, these time-series data were not used for formal inferential comparison among treatments over time. Instead, they are presented to illustrate general growth dynamics during early plant development.

Regression analysis for leaf area model development was conducted using the sampled compound leaves. The leaves used for regression analysis were independently sampled across all experimental units and treatments to capture broad variation in leaf morphology and minimize sampling bias associated with individual plants or cutting treatments. Candidate models were evaluated using coefficient of determination (R^2), significance of regression coefficients, and residual distribution patterns. To further assess predictive performance and model reliability, additional validation metrics, including Root Mean Square Error (RMSE) and Mean Absolute Error (MAE), were calculated for the selected model. Model robustness was also evaluated using 10-fold cross-validation, in which the dataset was randomly partitioned into ten subsets. In each iteration, nine subsets were used for model calibration and one subset was used for validation. The procedure was repeated until all subsets had been used for validation. Cross-validation performance was evaluated based on CV-R^2, RMSE, and MAE values. The selected model was therefore presented as the best-performing model for non-destructive leaf area estimation within the present study. All statistical analyses were conducted using R software (R version 4.4.1) through RStudio.

RESULTS AND DISCUSSION

Leaf area estimation

Regression analysis revealed a strong positive relationship between leaf length and leaf area of *M. oleifera* across all tested models (Figure 3). The data distribution indicated that leaf area increased with increasing leaf length, with a tendency toward a curvilinear pattern, particularly for larger leaves. The linear models (Figures 3.A and 3.C) produced coefficients of determination (R^2) of 0.8261 and 0.8257, respectively, indicating that approximately 82% of the variation in leaf area could be explained by leaf length. However, the power regression models provided slightly higher predictive accuracy, with R^2 values of 0.8453 and 0.8618 (Figures 3.B and 3.D). The best-fitting model among the single-predictor approaches was the power regression model using leaf width as the independent variable, with the equation $LA = 0.2489W^{1.9171}$, which accounted for 86.18% of the variation in leaf area. The exponent value close to 2 suggests a nonlinear increase in leaf area as leaf size increases. Overall, these results indicate that the power regression model is more representative than the simple linear model when using a single morphometric predictor.

When combined predictors were evaluated, the linear model based on leaf Length × Width (L × W) produced a substantially higher coefficient of determination ($R^2 = 0.9636$) and therefore represented the best-performing model within the present study. The relationship between L × W and the actual leaf area showed a strong positive correlation (Figure 4). Similarly, the linear regression model based on (L × W)/number of leaflets also showed high predictive performance, with an R^2 value of 0.9471. In contrast, the power regression models based on combined predictors exhibited lower predictive accuracy, with R^2 values of 0.8748 and 0.8139, respectively. To further evaluate model reliability, the selected zero-intercept linear L × W model was validated using 10-fold cross-validation. The cross-validation results demonstrated stable predictive performance, with a CV-R^2 value of 0.9628, RMSE of 57.85 cm^2, and MAE of 44.77 cm^2. These relatively low error values indicate that the model maintained high predictive capability across different subsets of the dataset and can reliably estimate leaf area using simple non-destructive morphometric measurements.

Overall, these results indicate that the linear model based on L × W provides the most accurate and practical approach for non-destructive estimation of leaf area in *M. oleifera* under the conditions of this study. Nevertheless, because the model was developed using data collected under a single greenhouse environment, further validation under different environmental conditions, developmental stages, and independent datasets would strengthen its broader applicability and robustness.

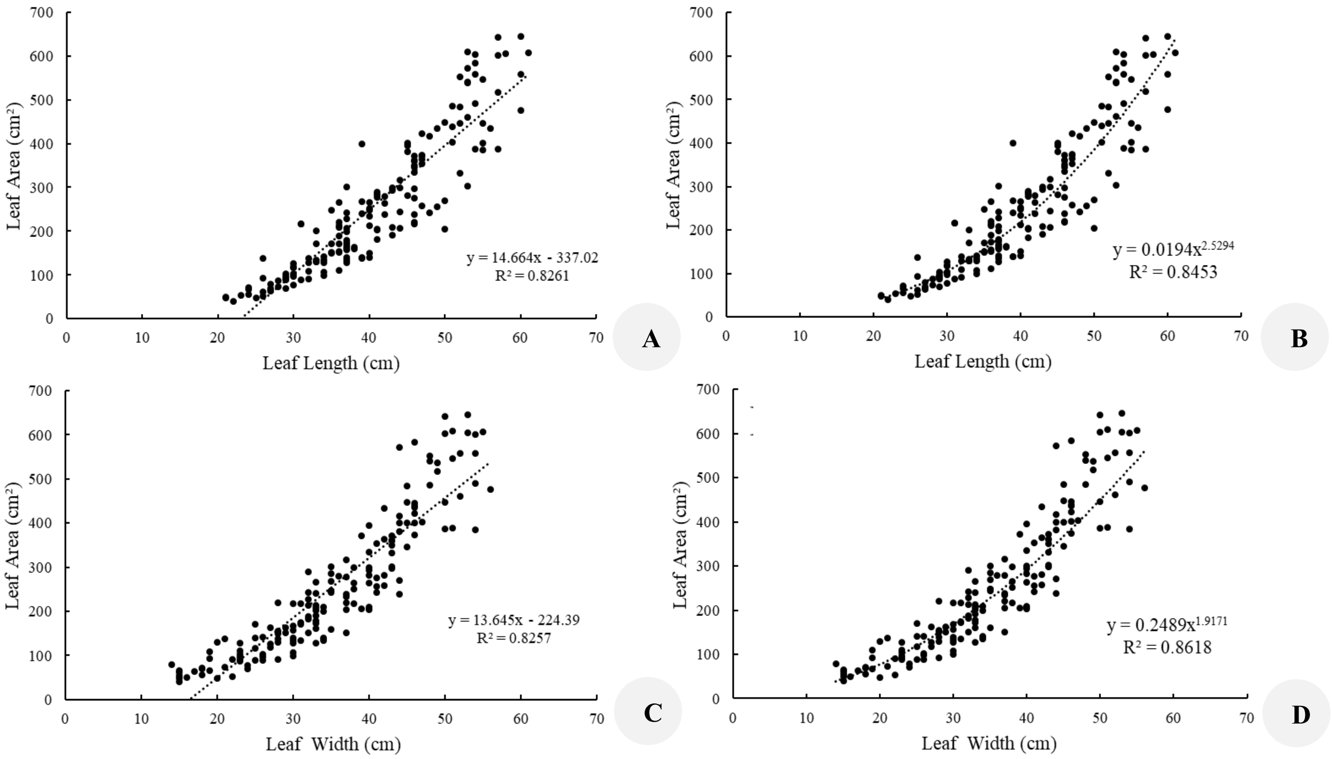

Figure 3. Comparison between linear model (left column) and power model (right column) for estimating the compound leaf area of *Moringa* using: A-B. Leaf length, and C-D. Leaf width as predictor

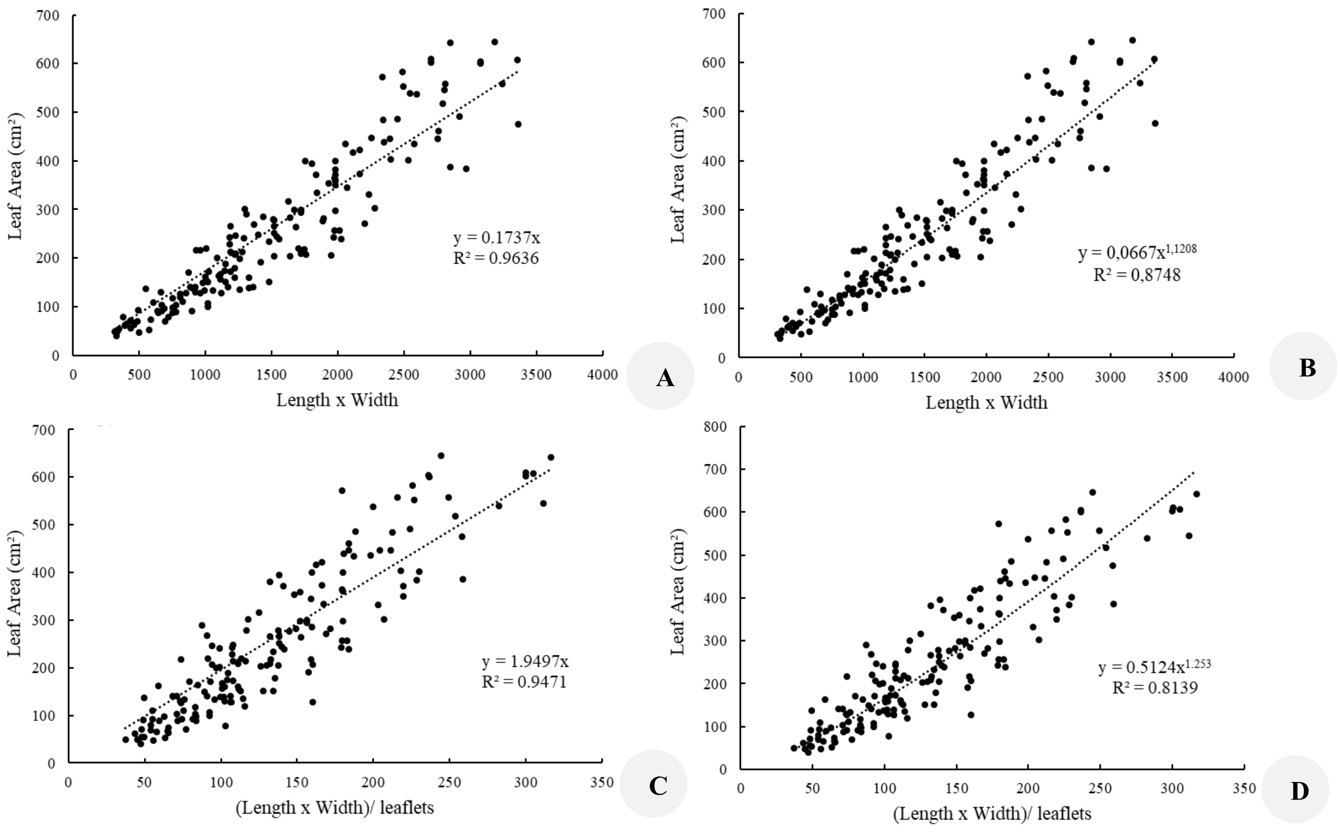

Figure 4. Comparison between the zero-intercept linear model (left column) and power model (right column) for estimating the compound leaf area of *Moringa* using: A-B. Leaf length × Width, and C-D. (Leaf length × Width)/number of leaflets as predictor

Early growth of *Moringa oleifera*

The survival percentage of *M. oleifera* stem cuttings varied across the different cutting lengths evaluated (Figure 5). Survival was calculated based on the total number of surviving plants relative to the initial number of cuttings per treatment (pooled across replications, n = 12 plants per treatment). The highest survival was recorded in P3 (40 cm) with 75.00%, followed by P2 (30 cm) and P1 (20 cm) with 54.16% and 45.83%, respectively. Longer cuttings showed lower survival, with P4 (50 cm) and P5 (60 cm) at 41.66% and 33.33%. Because survival estimates were derived from a relatively limited number of plants, these values should be interpreted cautiously and are presented only as descriptive observations rather than formal statistical comparisons. Small differences in surviving plant number may substantially influence percentage values under this sample size.

Stem cutting length showed different growth patterns in *M. oleifera* during the early vegetative stage, as reflected in the number of shoots, shoot length, number of branches, and number of leaves (Figure 6). The number of shoots increased gradually across all treatments, with P5 tending to produce more shoots at later observation periods, whereas P1 generally showed fewer shoots. In contrast, shoot elongation tended to be greater in P1 compared with longer cuttings. For branching and leaf production, P3 generally showed higher branch and leaf numbers during the observation period. However, because repeated-measures analysis was not conducted, these weekly patterns should be interpreted only as descriptive growth trends and not as evidence of statistically different temporal responses among treatments.

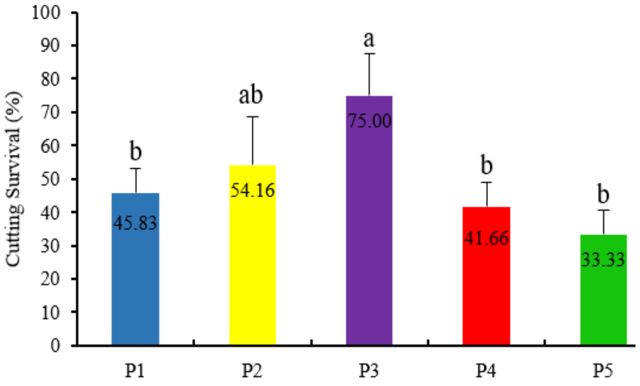

Figure 5. Survival percentage (%) of *Moringa oleifera* stem cuttings at different cutting lengths during early growth under greenhouse conditions. P1: 20 cm, P2: 30 cm, P3: 40 cm, P4: 50 cm, and P5: 60 cm stem cuttings

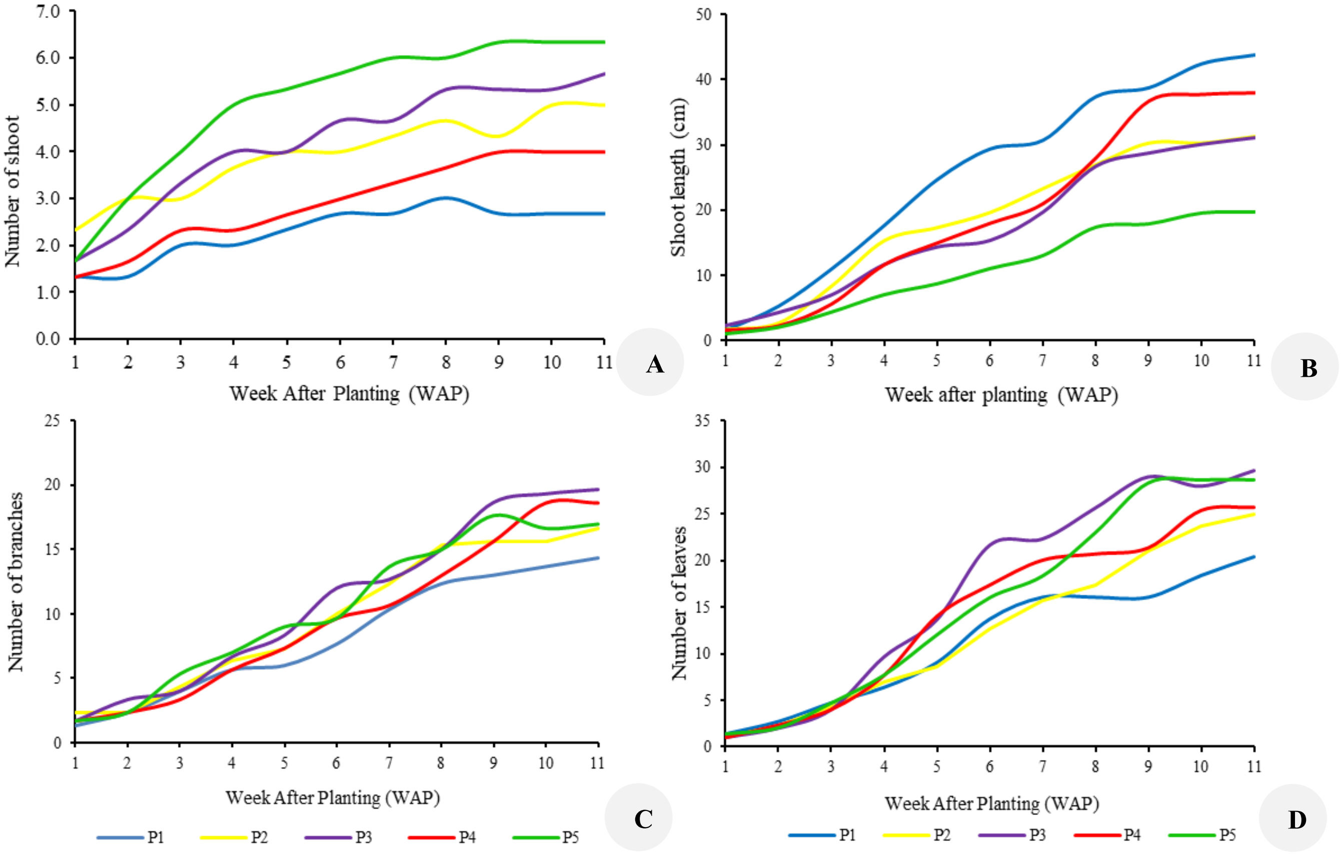

Figure 6. Effect of stem cutting length on vegetative growth of *Moringa oleifera*. A. Number of shoots, B. Shoot length, C. Number of branches, and D. Number of leaves during 1-11 Weeks After Planting (WAP)

Stem cutting length was associated with variation in several growth and root characteristics of *M. oleifera* (Table 1). SPAD value and canopy area were not significantly different among treatments. Canopy area showed relatively large standard deviations, particularly in P2 and P5, indicating substantial variability among individual plants during early establishment. This variation may reflect differences in branching pattern, canopy expansion rate, and survival among cuttings within the same treatment. Leaf area differed significantly among treatments, with P3 producing the highest mean leaf area (897.85 cm^2). Root length also tended to be greater in P3, although it was not significantly different from P1 and P4. For root diameter, P3 and P5 were grouped within the same Tukey category and therefore were not significantly different. These observations were consistent with the visual differences shown in Figure 7.

Biomass of *Moringa oleifera*

Stem cutting length did not significantly affect the Fresh Weight of Leaves (FWL), Stems (FWS), or Roots (FWR), nor the Dry Weight of Stems (DWS) in *M. oleifera* (Table 2). Fresh biomass values nevertheless showed numerical variation among treatments, with P3 tending to produce higher mean values in several parameters. Significant differences were observed in Leaf Dry Weight (DWL) and Root Dry Weight (DWR). The P3 treatment showed the highest mean DWL (3.25 g) and DWR (0.77 g), whereas the lowest DWR value was observed in P5 (0.31 g). Several biomass variables also exhibited relatively large standard deviations, suggesting considerable within-treatment variability during early growth. Because dry biomass is less influenced by short-term fluctuations in plant water content than fresh biomass, variation in DWL and DWR may provide a clearer indication of structural biomass accumulation during early establishment. The higher dry biomass values observed in P3 therefore suggest relatively greater biomass retention under greenhouse conditions, although physiological processes such as photosynthetic rate or carbon assimilation were not measured directly in this study.

Table 1. Growth and root characteristics of *Moringa oleifera* as affected by different stem cutting lengths

Treatments	SPAD value	Canopy area (cm^2)	Leaf area (cm^2)	Root length (cm)	Root diameter (mm)
P1	49.67±1.11a	292.71±165.66a	318.34±112.20c	33.00±3.61a	3.35±0.51bc
P2	49.23±3.64a	597.81±335.89a	588.02±63.82b	21.67±8.50a	2.79±0.70c
P3	54.43±8.31a	834.37±88.05a	897.85±159.75a	35.33±13.43a	7.61±1.29a
P4	53.76±4.57a	544.72±144.01a	585.14±34.37b	32.33±8.08a	5.81±1.17ab
P5	52.66±6.03a	641.04±478.80a	661.32±15.18b	14.00±1.00a	6.01±2.23a
MSD $_{0.05}$	16.31	850.98	236.38	24.99	3.76

Note: Data are presented as mean±standard deviation. Means within a column followed by the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$)

Table 2. Effect of stem cutting length on fresh and dry biomass of *Moringa oleifera*

Treatments	FWL (g)	FWS (g)	FWR (g)	DWL (g)	DWS (g)	DWR (g)
P1	4.90±2.75a	10.70±5.73a	2.23±0.81a	0.98±0.40b	2.04±1.22a	0.51±0.20a
P2	9.63±6.06a	10.43±6.40a	2.03±0.47a	1.87±1.03ab	1.93±1.27a	0.44±0.12a
P3	14.46±8.55a	14.06±7.14a	3.06±1.55a	3.25±1.75a	2.29±1.09a	0.77±0.44a
P4	7.00±1.57a	13.43±8.75a	1.73±0.61a	1.61±0.28ab	2.33±1.50a	0.40±0.14a
P5	8.73±4.99a	10.23±4.50a	1.73±0.67a	1.71±1.35ab	1.96±0.64a	0.31±0.09b

Note: Data are presented as mean±standard deviation. Means within a column followed by the same letter are not significantly different according to Tukey's HSD test ($p \leq 0.05$). Fresh Weight of Leaves (FWL), Fresh Weight of Stem (FWS), Fresh Weight of Root (FWR), Dry Weight of Leaves (DWL), Dry Weight of Stem (DWS), and Dry Weight of Root (DWR)

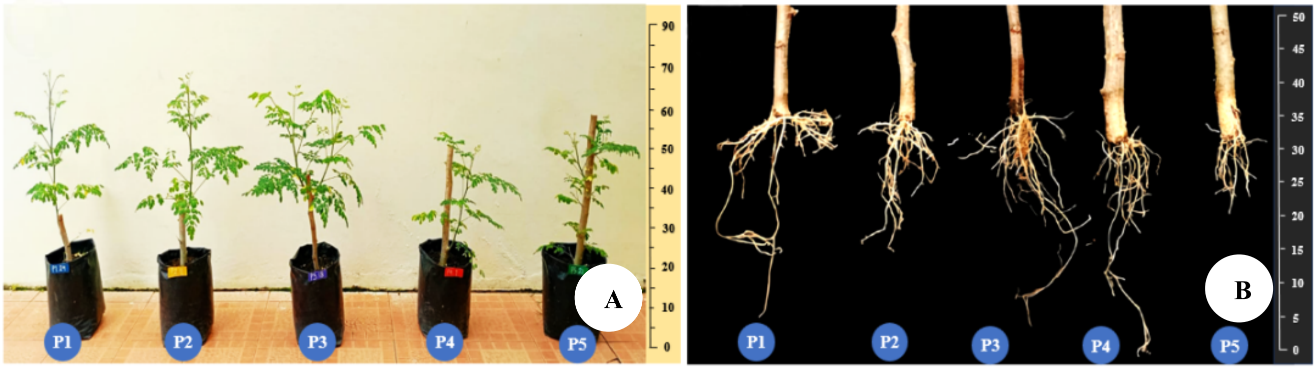

Figure 7. Growth and root morphology of *Moringa oleifera* under different stem cutting lengths with: A. Plant growth, and B. Root morphology and root system development. P1: 20 cm, P2: 30 cm, P3: 40 cm, P4: 50 cm, and P5: 60 cm

Discussion

Non-destructive estimation of leaf area in Moringa oleifera

Leaf area is widely recognized as a key indicator of plant growth because it determines the effective photosynthetic surface available for light interception and carbon assimilation. Leaves function as the primary organs for capturing solar radiation, facilitating gas exchange, and producing assimilates through photosynthesis. Consequently, increases in leaf area are generally associated with greater biomass production potential. In *M. oleifera*, which has compound leaves composed of numerous leaflets, increases in leaflet size and number can collectively expand the canopy photosynthetic surface. This highlights that leaf area serves not only as a morphological descriptor but also as a functional indicator of canopy development and vegetative productivity (Gustiar et al. 2023a; Quijano and Morandi 2023). Previous studies further indicate that variation in canopy architecture, leaflet morphology, and growth performance among *Moringa* genotypes is closely related to leaf production and yield (Albaugh et al. 2020; Muniandi et al. 2024; Zeru et al. 2025).

The present study showed that the linear model based on leaf Length × Width (L × W) produced the highest predictive performance among the evaluated equations. The present study showed that the linear model based on leaf Length × Width (L × W) produced the highest predictive performance among the evaluated equations. This finding indicates that combining longitudinal and lateral leaf dimensions provides a more representative approximation of compound leaf expansion than the use of a single predictor alone. Comparable findings have also been reported in other compound-leaf species. Lakitan et al. (2022) observed that combined morphometric variables improved leaf area prediction accuracy in cassava, while Gustiar et al. (2023b) reported a similar response in chaya, where models based on combined leaf dimensions produced stronger predictive relationships than single-variable models. These previous findings support the hypothesis that two-dimensional morphometric approaches are more suitable for species with complex leaf architecture.

The strong performance of the L × W model may be associated with the ability of both variables to jointly represent rachis elongation and lateral leaflet expansion. However, this explanation remains morphology-based and was not directly validated through anatomical or developmental analysis in the present study. Several limitations should also be considered when applying this model. The estimation approach is based on measurements of whole compound leaves, whereas leaflet number, arrangement, and spatial distribution may vary depending on developmental stage, genotype, and environmental conditions. Such variation can influence the relationship between L × W and actual leaf area, potentially affecting model accuracy outside the calibration conditions of this study. Therefore, caution is needed when applying the model across different growth stages or environments, and further validation under diverse conditions is recommended to confirm its general applicability.

Effect of stem cutting length on vegetative propagation and early growth of Moringa oleifera

The present study demonstrated that stem cutting length influenced several vegetative growth characteristics of *M. oleifera*, particularly leaf area, branch number, root morphology, and dry biomass accumulation. Cuttings of 40 cm generally showed more favorable growth responses compared with shorter or longer cuttings. Previous studies have reported that larger cuttings may contain greater carbohydrate reserves and more active nodes, which can support shoot emergence and early establishment (Otiende and Maimba 2020; Muniandi et al. 2025). These findings support the hypothesis that differences in reserve availability may contribute to the variation in growth responses observed among cutting lengths in the present study.

The observed responses may also be conceptually related to endogenous hormonal regulation during adventitious root formation and shoot development. Auxin is generally associated with root initiation, whereas cytokinin promotes shoot development (Sahoo et al. 2021; Sosnowski et al. 2023). However, endogenous hormone concentrations were not quantified in this experiment; therefore, these physiological explanations remain speculative and should not be interpreted as direct experimental evidence.

The growth trajectories observed in Figure 6 indicate a trade-off between shoot number and shoot elongation. Longer cuttings, particularly P5, tended to produce more shoots during the observation period, whereas shorter cuttings, such as P1, generally produced fewer but longer shoots. This pattern is consistent with the possibility that longer cuttings provide more potential sites for shoot initiation, while shorter cuttings may allocate available reserves more intensively toward elongation of fewer shoots. Similar shoot-elongation trade-offs have been described in vegetative propagation studies of woody perennial species (Pamungkas et al. 2021; Bektas et al. 2023). In the present study, 40 cm cuttings also produced relatively greater branch and leaf numbers, indicating more balanced canopy development during early growth. Previous studies have reported that increased branching and canopy expansion are associated with greater leaf production in *Moringa* (El Bilali et al. 2024; Gustiar et al. 2025). The observed response may indicate that moderate cutting length supports more balanced canopy development during early vegetative growth. However, physiological mechanisms underlying this response were not directly evaluated in the present study.

Root development also varied among treatments, with 40 cm cuttings showing relatively greater root length and diameter. A more developed root system is generally associated with improved water uptake and plant establishment during early growth stages. Nevertheless, substrate moisture dynamics and nutrient availability were not separately evaluated. therefore, the mechanisms underlying root development differences remain uncertain.

Although fresh biomass parameters did not differ significantly among treatments, differences were observed in leaf and root dry weights. Dry biomass is less influenced by plant water content and may reflect differences in structural biomass accumulation more consistently than fresh biomass. The higher dry biomass observed in the 40 cm treatment suggests relatively greater biomass retention during early establishment. However, physiological parameters such as photosynthetic rate or carbon assimilation were not directly measured in this study.

Overall, the results suggest that stem cutting length plays an important role in early vegetative development of *M. oleifera*. Cuttings of approximately 40 cm showed favorable performance across several growth parameters under the conditions of this study. These findings have practical implications for nursery management, particularly in selecting appropriate cutting size for vegetative propagation. However, these interpretations are based on greenhouse conditions and should be validated under broader environmental settings.

In conclusion, this study demonstrated that leaf morphometric parameters can be effectively used for non-destructive estimation of leaf area in *M. oleifera*. Among the evaluated models, the linear regression based on leaf Length × Width (L × W) produced the highest predictive performance within the calibration dataset used in this study. The results also showed that stem cutting length influenced several early vegetative growth characteristics, particularly leaf area, root morphology, and dry biomass accumulation during greenhouse establishment. Cuttings of approximately 40 cm generally showed more favorable overall early-growth responses, particularly in survival, leaf area, root development, and dry biomass variables. These findings suggest that moderate cutting length may support balanced early plant establishment in *Moringa* propagation systems under greenhouse conditions. This study contributes by integrating vegetative propagation evaluation with a calibrated non-destructive leaf area estimation approach within the same experimental framework, thereby providing a practical tool for repeated seedling growth monitoring in nursery systems without destructive sampling. However, because this study was conducted under greenhouse conditions and the regression model was not externally validated, caution is needed in applying these results more broadly. Further studies involving independent model validation, repeated testing under field conditions, and evaluation across different genotypes or environments are recommended to confirm the wider applicability of the proposed approach.

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