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Accurate and non-destructive estimation of palmate compound leaf area in cassava (*Manihot esculenta* Crantz) based on morphological traits of its selected lobes

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Abstract Results indicated that total area of cassava compound leaf showed the best-predicted ($R^2 = 0.9847$) using LW of middle lobe using the zero-intercept linear regression; for faster and simpler data collection, L of middle lobe was selected as predictor and the power regression was utilized in estimation of LA ($R^2 = 0.9299$); a more complicated predictor using average LW of middle, left-most, and right-most lobes combined with number of lobes (NoL) did not significantly increase accuracy ($R^2 = 0.9882$) compared to that using LW of middle lobe as predictor. L/W ratio was more consistent in large lobes, especially for the middle lobes (STD = 0.259). Model validation assured that all predictors combined with each appropriate models were reliable; therefore, they are recommended for estimating LA of the cassava leaf.

Keywords: Compound leaf; Leaf area estimation; *Manihot esculenta*; Symmetric lobe; Zero-intercept regression

Introduction

Cassava (*Manihot esculenta* Crantz) is an easy-to-grow plant in the tropical lands, including on low nutrient soil and low annual rainfall condition. Roots of cassava are a source of carbohydrate for food, feed, bioethanol for energy (Krajang *et al*., 2021; Marx, 2019), and raw material for agroindustry, including bioplastics (Zoungranan *et al*., 2020). Leaves of cassava are commonly consumed as green vegetable (Okareh *et al*., 2021).

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It is a perennial crop. However, farmers also commonly harvest the young leaves for being used as vegetable while waiting for root harvesting (Hauser *et al*., 2021). There are also some farmers who fully dedicate their cassava crops for harvesting as leafy vegetable. Either ways, farmers must manage their leaf harvesting such that root yield could be maintained at acceptable level.

Marketable leaves of cassava can be harvested at 90 days after planting (DAP) until roots were harvested. Optimum leaf yield was produced during 120 to 150 DAP (Pipatsitee *et al*., 2019). Leaf harvesting in 2 to 4 weeks had insignificant impact on production of cassava roots. The amount of harvested cassava leaves per plant should be reasonable for maintaining growth and yields (Munyahali *et al*., 2017). Leaf is the source of assimilates, manufactured via photosynthesis, and then translocated to storage organ, such as roots in cassava (Karim *et al*., 2010).

Considering the vital role of the leaves, a non-destructive LA estimation models based on allometric measurements is clearly useful for monitoring leaf enlargement rate (Nabila and Noer, 2018) and for estimating leaf yield at any time needed.

The objective of this study was to develop leaf area (LA) estimation models in cassava plant which is accurated, easy to do and affordable by farmers.

Materials and methods

Description of the cassava leaf

Cassava used in this study was Adira-1. Its compound leaf consists of 5 to 9 cuneate type lobes. The lobes are palmately arranged. Each lobe has attenuated base and cuspidated tip. Edge of lobes and the whole leaf is entirely smooth. Reticulated venation consists of small veins forming a network throughout the leaf blade. Each lobe has a midrib emerged form petiole-blade junction straight to tips of each lobe (Figure 1).

The compound leaves of cassava used in this study were dominantly (62.75 %) consisted of 7 lobes and were rarely (11.75 %) consisted of even-number lobes, i.e., 6 or 8 lobes. The size and shape were not similar between middle and left-most or right-most lobe. The middle lobe was larger and symmetric in shape; meanwhile, both left-most and right-most lobes were smaller and asymmetric between left and right sides (separated by the midrib) of their blade. Blade shape of the most-left lobe was mirrored by the most-right lobe, yet their sizes can be different. Middle, left-most, and right-most lobes, as predictors, were tested for accuracy for estimating the whole area of cassava compound leaf. In case of the even number lobe, the two middle lobes were averaged. The average represented the value of middle lobe in development of LA estimation model.

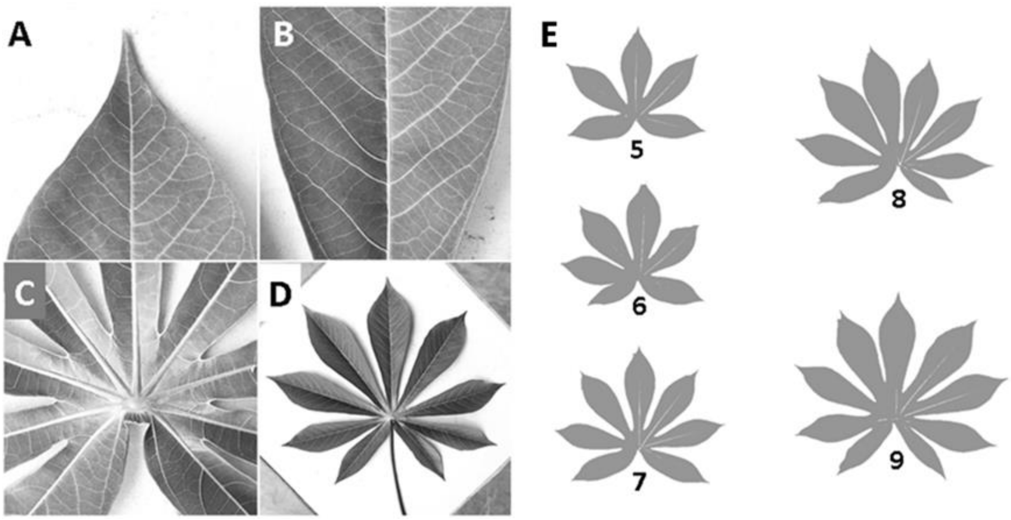

Figure 1. Parts and number of cuneate lobes in the palmate compound cassava leaf. A = lobe tip; B = lobe mid-section; C = center part of the compound leaf blade, connected to petiole; D = arrangement of the palmate compound leaf; and E = the silhouette of leaf with 5 to 9 lobes

Model development and validation

Data collection was carried out in the morning to keep the leaves in fresh condition during measurements. Leaves were purposively selected to maximized range of size and ensured even distribution of leaves used in developing LA estimation models. However, leaves used in validating the models were randomly picked within lower, middle, and upper segments of cassava canopy. This approach was chosen to ensure that any leaf sizes were well represented in the models; moreover, the leaf sizes randomly picked for LA estimation were also within the range covered by the developed model.

Effective predictors and type of regressions have been identified from some previous studies (Lakitan *et al*., 2021; Meihana *et al*., 2017; Widuri *et al*., 2017). Selected predictors were L, W, LW, and number of lobes (NoL). Adopted models were zero-intercept linear and power regressions. Direct measurement of LA was done by using the automated digital image analysis application developed by Easlon and Bloom (2014).

The use of LW as predictor for LA estimation using linear regression model has been proven accurate and reliable in many simple leaf with single blade, but it might consist of leaves with different shapes. Since shape of middle and side leaves are slightly different; then, it was necessary to test which lobe amongst left-most, middle, and right-most lobes has higher accuracy (R^2 value) in estimating total area of cassava compound leaf.

Three steps were taken. The first one was to use LW of the favorable middle lobe as predictor and adopting the zero-intercept linear regression model for predicting LA of the cassava compound leaf. The second step was to evaluate further possibility of using single trait, i.e., L or W of the best lobe for quick estimation of the compound leaf area. Since increase in L of lobe always proportionally followed by increase in its W and vice versa; therefore, the appropriate model used was power regression or second-order polynomial regression for estimating LA, not the linear regression. The third step was exploring possibility of increasing accuracy and reliability by adding more traits into the already-accurate model using LW as predictor. The additional trait was number of lobes (NoL) per compound leaf in cassava plant. Instead of using only LW of the middle lobe; or the average of middle, left-most, and right-most lobes; or adding NoL on top the average LW of all represented lobes (LW*NoL) was used as predictor.

Statistical analysis

Models used were zero-intercept linear regression, LA = β LW and LA = β LW*NoL, α was the intercept and set at zero, and β was slope; and power regression, $LA = aL^b$ or $LA = aW^b$, a and b were the coefficients that describe the relationship between L and LA or W and LA, respectively. The coefficient of determination (R^2) indicates the accuracy level of each developed model. The R^2 value between measured and predicted LA, indicated level of reliability of the model.

Results

Results indicated that middle lobe performed better that both left-most and right-most lobes in estimating LA in cassava plant (Figure 2). The accuracy comparison was conducted using LW as predictor and zero-intercept linear regression model. Coefficient of determination (R^2) was used as proxy of accuracy. The R^2 value of middle lobe was 0.98 and for left-most and right-most lobes were 0.88 and 0.89, respectively. Better R^2 value if the middle lobe was associated with uniform shape of the middle lobe. This result created possibility to accurately estimate compound LA of cassava by single allometric measurement of length (L) or width (W) of middle lobe.

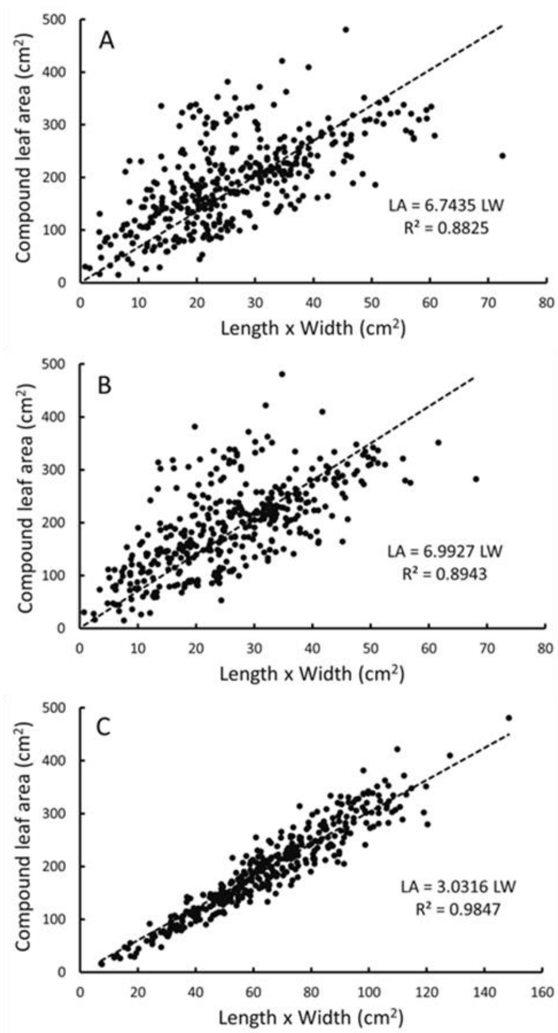

Figure 2. Comparison amongst left-most (A), right-most (B), and middle (C) lobes on the leaf area estimation in cassava (*Manihot esculenta*), if length x width (LW) was used as predictor and the zero-intercept linear regression was adopted as the model

L or W of middle lobe of compound cassava leaves was evaluated for simplifying allometric measurement while maintaining accuracy of LA estimation. Leaf growth is three dimensional, i.e., L, W, and thickness. Since thickness of cassava leaves was less than 1 mm, measurement of LA was focused on the other two-dimensional surface of L and W. Naturally, increase in L is proportionally followed by increase in W and vice versa; therefore, relationship between L or W and LA is not linear. In this study, power regression model was used for estimating LA. Meanwhile, L or W was used as predictor (Figure 3).

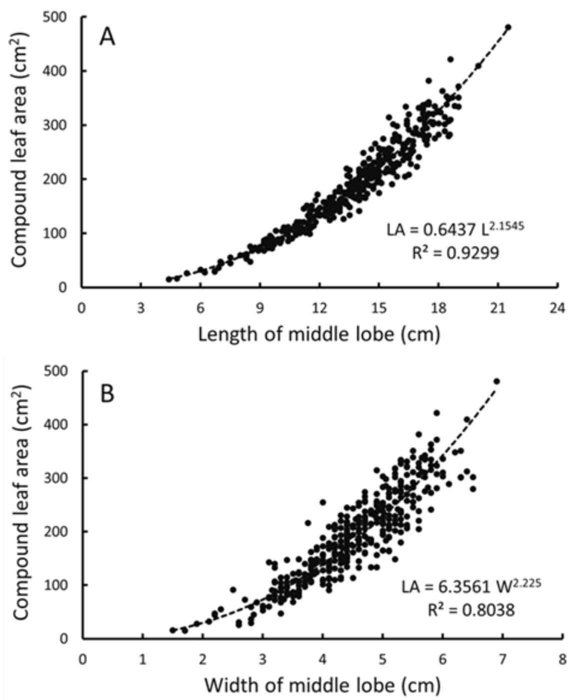

Figure 3. Comparation between length (A) and width (B) of the middle lobes as predictors of leaf area estimation using the power regression model in cassava (*Manihot esculenta*)

Based on the R^2 value, L of middle lobe (R^2 = 0.93) was a better predictor than W of middle lobe (R^2 = 0.80). Estimation of compound leaf area in cassava was more accurate and reliable in predicting LA if LW was used (R^2 = 0.98) than if L of middle lobe was used (R^2 = 0.93) as predictor. LW covered probable variation on both length and width. Meanwhile, variability of predicted LA was sensitive to the dynamic variation in width of middle lobes. Length of the middle lobe was recommended for quick data collection since the R^2 value was also acceptable. Increasing relevant number of traits incorporated into the predictor was expected to improve accuracy and/or reliability of the LA estimation in compound leaf.

The R^2 sequentially increased as number of component predictors was systematically added from 2 predictor components of LW average of left-most and right-most lobes (R^2 = 0.91), increased to 3 components by adding the LW average of the middle lobe (R^2 = 0.97), and finally, to 4 components by multiplication of all three LW averages to number of lobes per compound leaf (R^2 = 0.99). The zero intercept linear regression models were used to estimate total area of the compound leaf in cassava plant (Figure 4).

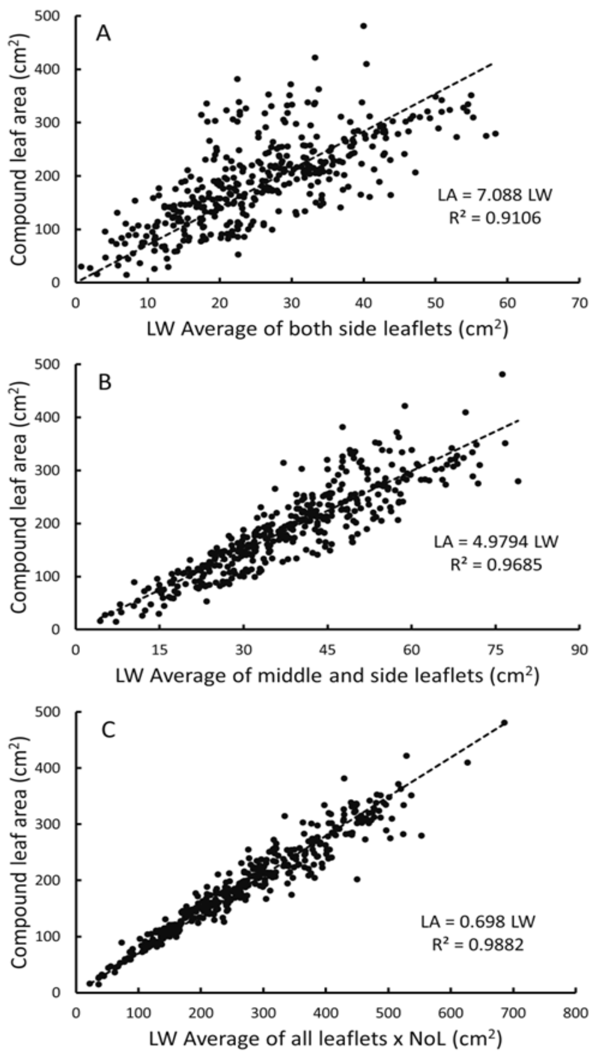

Figure 4. Coefficient of determination (R^2) using LW average of both side lobes as predictor (A), increase of the R^2 by adding middle lobe (B), and by multiplying with number of lobes per compound leaf (C) in cassava (*Manihot esculenta*)

Similar trend was observed if the power regression model was used. Use of single trait, i.e., length of the left-most lobe alone, was not adequate (R^2 = 0.57) for estimating an area of compound leaf in cassava; however, use of 2 traits, i.e., both left-most and right-most lobes, improve accuracy (R^2 = 0.71) in estimating the cassava leaf area. Further, use of 3 traits by adding the length of middle lobe further increased accuracy (R^2 = 0.81). The highest accuracy (R^2 = 0.91) was achieved after average length of all three left-most, right-most, and middle lobes were multiplied with number of lobes per compound leaf (Figure 5).

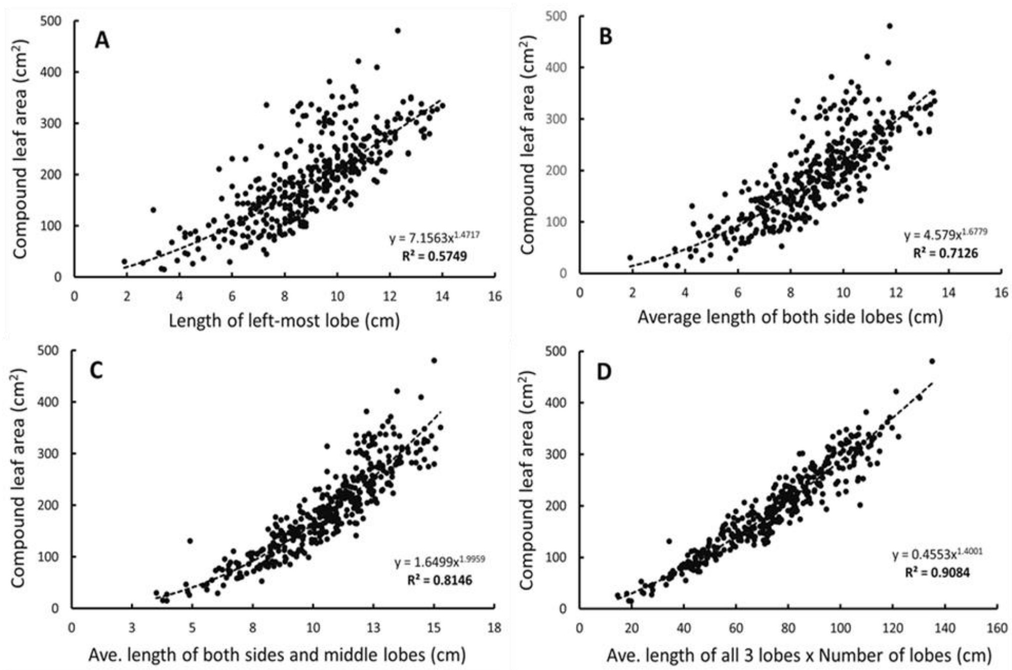

Figure 5. Addition of relevant leaf traits increases accuracy of leaf area estimation in cassava (*Manihot esculenta*)

Basically, there were three complementary traits used for estimating area of compound leaf in cassava plant, i.e., length of lobe (L), width of lobe (W), and number of lobes per compound leaf (NoL). All developed models combined with all traits or combination of traits used as predictors can be routinely validated; however, only selected high performance combination of models and each representation of 1-trait (L), 2-trait (LW), and 3-trait (LW*NoL) predictors were presented in Figure 6. The selected predictors were length of middle lobe, LW of middle lobe, and LW*NoL of middle lobe multiplied with number of lobes per compound leaf. The validation used different population of compound leaf but of the same cassava cultivar. All selected models were successfully validated (R^2 > 0.98) and classified as reliable models.

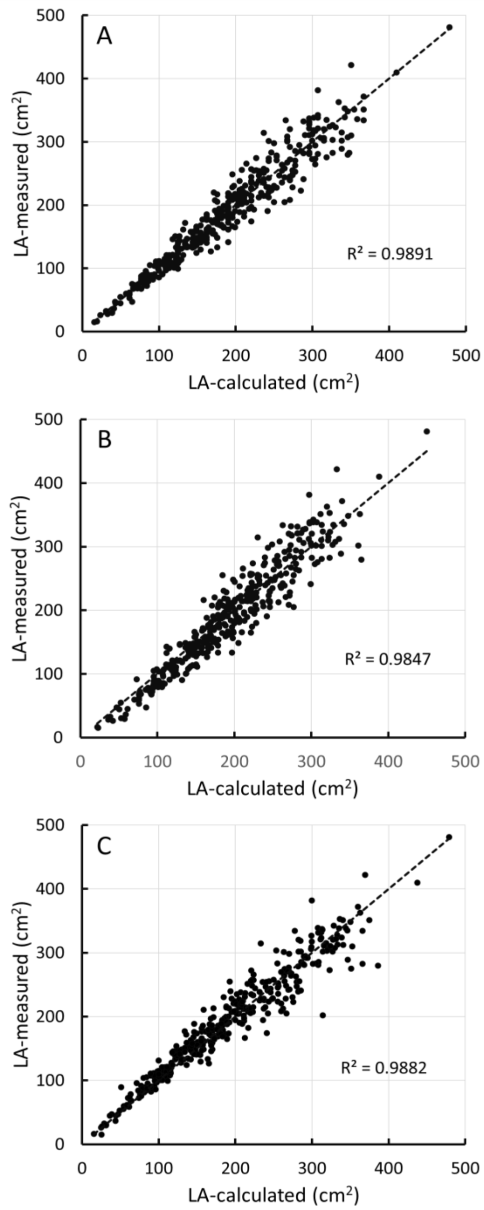

Figure 6. Validation of the models using length of middle lobe (A), LW of middle lobe (B), and averaged LW x number of lobes per compound leaf (C) as predictors

The L/W ratio of middle lobe was lower than that of side lobes as visually exhibited in Figure 7, indicating different dimension between middle and side lobes, i.e., middle lobe was slimmer than side lobes.

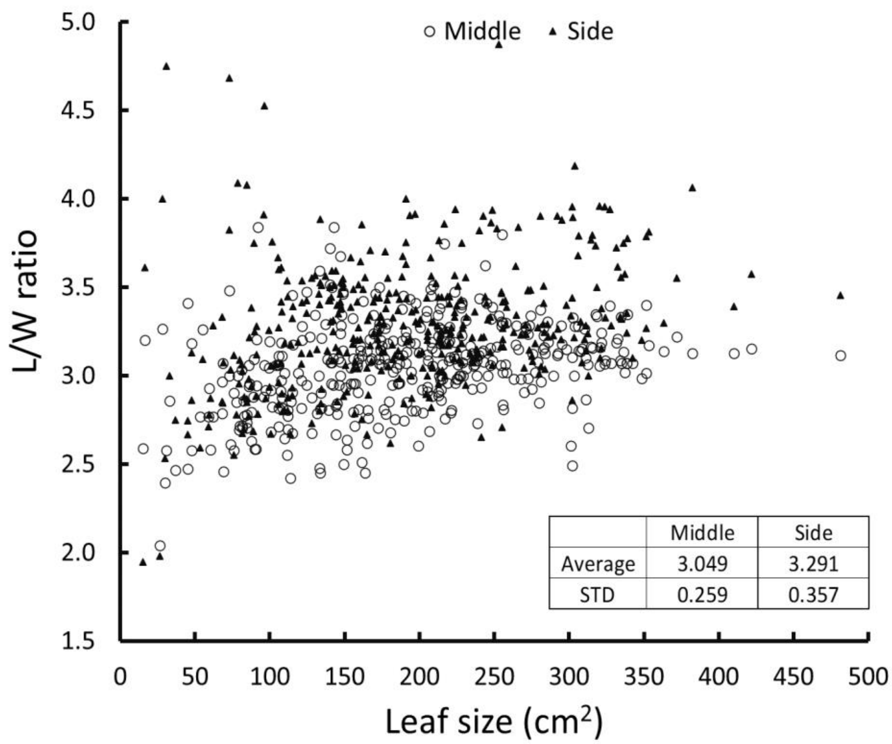

Figure 7. Visual comparison of the L/W ratio across leaf size between middle and side lobes in cassava (*Manihot esculenta*)

Discussion

Results of this study indicated that the middle lobe was a better predictor than left-most or right-most lobe, the length was a better predictor than width of the middle lobe, the multiple relevant traits was better predictor than single trait, the length of middle lobe, LW of middle lobe, and LW*NoL were all reliable predictors with R^2 = 0.9891, 0.9847, and 0.9882, respectively and L/W ratio was more consistent in large lobes, especially in middle lobes (STD = 0.259) compared to side lobes (STD = 0.357). Shi *et al*. (2019) reported that the best model for estimating LA was the one developed based on proportional relationship between LW and LA. Models that relate LW to LA have been reported in many plants, including chili pepper (Widuri *et al*., 2017); *Crotalaria juncea* (Carvalho *et al*., 2017); tomato (Meihana *et al*., 2017); chia (Mack *et al*., 2017); *Limnocharis flava* (Lakitan *et al*., 2018a); and olive (Koubouris *et al*., 2018). Lakitan *et al*. (2021) reasoned that LW was a directly related and geometrically-sound predictor since LW was basically an imaginary rectangular area created based on length (L) and width (W) of the estimated leaf. Each estimated LA is in a perfect fit within the imaginary LW rectangle. The area of LW is instantly adjusted to changes in L and W. This study had moved a step further, i.e., searching for effective traits in lobe as a predictor for estimating full area of the compound cassava leaf.

Successful in developing LA estimation model had been achieved for trifoliate leaf of common bean (*Phasolus vulgaris*) using LW of terminal leaflet as predictor and adopting the zero-intercept linear regression model. However, LW of terminal leaflet could not be used for estimating total area of the bipinnate compound leaf in celery (*Apium graveolens*). Instead, LA of the celery compound leaf can be estimated using L (based on lengths of terminal leaflet plus rachis) and W (based on total tip-to-tip distance between pair of the two longest side leaflets). This specified LW as predictor and the zero-intercept linear regression were used in developing LA estimation model for leaf celery (Lakitan *et al*., 2021).

Cassava compound leaf consists of 5 to 9 cuneate shape lobes. The lobes were arranged in digitate form. Lobes at three specific locations, i.e., left-most, middle, and right-most lobes, were selected as candidate for predictors. The two middle lobes were averaged in case of leaf has an even number (6 or 8 lobes). The middle lobe was a better predictor (R^2 = 0.9847) than the left-most and right-most lobes. The middle lobe exhibited more consistent symmetrical cuneate shape and as the largest lobe among all other lobes in each leaf used.

In case of only single linear measurement of either L or W was used as predictor, quadratic and power regression models showed better R^2 values than linear regression model. L of the middle lobe was a better predictor than W. Length of the middle lobe alone can be used as an accurate predictor (R^2 = 0.9299) for LA. Koubouris *et al*. (2018) also found that LA was estimated with higher accuracy by employing L alone, as compared to W alone in eight olive cultivars studied. Similar findings were reported by Widuri *et al*. (2017) in chili pepper using second-order polynomial or power regression. However, the R^2 was higher if W was used as predictor instead of L in estimating LA in tomato (Meihana *et al*., 2017).

It was found that in predicting LA of the digitate-compound cassava leaf, L of middle lobe was a better predictor than W with R2 = 0.9299 and 0.8038, respectively. Zanetti *et al*. (2017) also found that L of middle lobe was an accurate (0.92) predictor for LA estimation. Therefore, for quick yet accurate LA estimation of the cassava compound leaf, it is recommended to use the length of middle lobe as predictor and using power regression as the appropriate model.

In spite of the consistent findings that LW was a more reliable predictor than L or W separately (Ghoreishi *et al*., 2012; Giaccone *et al*., 2017; Koubouris *et al*., 2018; 2021; Pompelli *et al*., 2012); many had experimented to modify the simple LW into different and more complicated combination of L and W, including LLxLW (Mack *et al*., 2017), $LW_{0.5}$ (Ghadami-Firouzabadi *et al*., 2015), or L^2W and LW^2 (Hinnah *et al*., 2014); or introducing additional predictors on top of the basic L-W combination, including petiole length and leaf shape traits (Fanourakis *et al*., 2021) and leaf fresh and dry weight (Huang *et al*., 2019).

Adding relevant traits to an initial single trait predictor may increase accuracy and reliability of LA estimation; reversely, addition of the less related traits may decrease the accuracy. Adding a characteristically different but complementary trait(s) can increase accuracy of LA estimation. For instance, adding leaf width to pre-established leaf length increased accuracy from R^2 = 0.9299 to R^2 = 0.9847 and adding number of lobes per leaf to pre-established average length of left-most, middle, and right-most lobes, increased R^2 from 0.8145 to 0.9078).

The use of the most appropriate single trait predictor can result in a more accurate LA estimation than combination of many-but-less relevant traits. For example, use of middle lobe LW was more accurate (R^2 = 0.9847) than use of average LW of left-most, middle, and right-most lobes (R^2 = 0.9685). Similar case exhibited a higher accuracy (R^2 = 0.9299) of LA estimated using length of middle lobe as single-trait predictor than using average length of left-most, middle, and right-most lobes as a multiple-trait predictor (R^2 = 0.8149). These findings lead to a hypothesis that the use of single closely related trait to leaf morphology, as the predictor in estimating LA, can be more accurate than using multiple-but-less-relevant traits.

Fanourakis *et al*. (2021) found that the use of the leaf shape traits in predicting LA generally led to poor LA estimations. LA was more related to leaf fresh weight than leaf dry weight (Huang *et al*., 2019). This implied that measured LA was dependent on leaf water content. Non-destructive leaf area estimation using spectral reflectance model under water deficit condition in cassava had been studied by Pipatsitee *et al*. (2019). Leaves were intentionally selected to maximized range of leaf size and ensured even distribution of leaves used in developing LA estimation models. However, leaves used in validating the models were randomly picked. This approach was used to ensure that any leaf size was well represented, and leaf sizes used for LA calculation were within the range covered by the developed model; therefore, there is no extrapolation. The approach yielded significantly high R^2 value for each validated models were 0.9891, 0.9847, and 0.9882 for length of middle lobe, LW of middle lobe, and averaged LW*NoL per compound leaf used as predictors, respectively.

Lower STD value of the L/W ratio indicated that the lobe shape was more uniform, as the case of middle lobe in cassava leaves. More uniform lobe shape led to a better trait of middle lobe as predictor of LA over either left or right lobe. Declining STD value and steady L/W ratio as the leaf growing implied that an evolutionary stability for the leaf shape toward large/older leaves. Ratio of LA/LW ranged between 1/2, which corresponds to a triangular leaf with leaf length as its height and leaf width as its base, and $\pi/4$, which corresponds to an elliptical leaf with leaf length as its major axis and leaf width as its minor axis (Shi *et al.*, 2019). Shape of middle lobe of cassava leaf is symmetric and very consistent but left-most and right-most lobes are non-symmetric and inconsistent. Deviation of leaf shape from its regular shape was more frequently observed in small/young leaves.

Accurate estimation of LA in cassava plant can be achieved using zero-intercept linear regression model with LW of middle lobe as predictor. Quick yet acceptable LA estimation can be done using power regression model with only using single morphological trait of middle lobe length as predictor. Additional traits beyond LW for increasing accuracy should be focused on dimension-related traits of the leaf blade or parts of the compound leaf. Reliability of models for estimating LA can be increased by maximizing range of leaf sizes and evenly distributing the leaves with different sizes during development of the models. L/W ratio can be used as indicator of leaf shape deviation.

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