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Non-destructive leaf area estimation in habanero chili (*Capsicum chinense* Jacq.)

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Abstract The best fit models were polynomial (R_L^2=0.9731; R_w^2=0.9620) and power (R_L^2=0.9692; R_w^2=0.9592) regressions if single predictor of leaf length (L) or width (W) was used. Meanwhile, if LW was used, the best fit models were the zero-intercept linear (R_{Lw}^2=0.9929) and power (R_{Lw}^2=0.9962) regressions. Forcing the intercept to zero yielded better estimation for smaller leaves and did not significantly alter the coefficient of determination. Configuration of scattered data helped to recognize the curving trend and should be used as reference in selecting an appropriate regression type. The second-order polynomial regression curve has a single bend, therefore, far-end of the curve would either rise to infinity or curve down after a rising start. These both cases are an inherited weakness of the second-order polynomial regression beyond range of collected data. Both problems associated with the decrease of leaf area (LA) at higher predictor values and under-estimating of LA at lower predictor value were successfully eliminated by opting to use the power regression if L^2W or LW^2 as predictors. Accuracy and reliability of separated L (R^2=0.9843) or W (R^2=0.9899) was lower than combined LW (R^2=0.9960) as predictor in case of habanero chili. Significant differences in specific leaf fresh weight (SLFW) and leaf water content (LWC) between young leaves and mature leaves should be recognized as a source of discrepancy before considering using weight-related traits in developing LA estimation model. Use of 120 to 160 regularly-shape leaves were sufficient for creating an accurate LA estimation if the selected leaves were evenly distributed and covering wide range of leaf size in habanero chili.

Keywords: Dimension-related trait; Leaf shape; Model validation; Weight-based predictor; Zero intercept regression

Introduction

The leaf area (LA) is a useful and widely used morphological trait. Further, the LA estimation model provides advantage over the destructive LA measurement approach albeit the later using sophisticated and expensive instruments, such as the digital leaf area meter. Destructive approach limits LA measurement to only once because of the targeted leaf must be cut off.

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Meanwhile, LA estimation model using leaf length (L), leaf width (W), or the product of L and W (LW) enables non-destructive and repeatable measurements over desired period. Growth analysis traits can be collected at any times on each of the assigned leaves. Availability of LA data enables for calculating the absolute and relative leaf expansion rates (Meihana *et al.*, 2017; Widuri *et al.*, 2017), maximum and average size of leaves exposed to abiotic stresses, L/W ratio as indicator of shape changing during leaf growth, and many other traits calculated based on leaf morphological traits.

The advantages of LA estimation model include easy to calculate, accurate, cost-effective, less dependent of sophisticated instrument, and non-destructive method for studying plant growth and development (Buttaro *et al.*, 2015; Khan *et al.*, 2016; Cirillo *et al.*, 2017; Lakitan *et al.*, 2017; Teobaldelli *et al.*, 2019). However, the recent LA estimation models do use modern digital-base instrument or sophisticated application in collecting required data for development of the models. The use of sophisticated instrument or application boosts accuracy and increases reliability of based data for later use in developing LA estimation models. Allometric data were collected on selected predictors (Guan *et al.*, 2020; Sabouri *et al.*, 2021; Tu *et al.*, 2021).

Many leaf morphological traits have been used as predictors for LA estimation. Use of multiple predictors occasionally results in higher coefficient of determination (R^2) yet repeatability in different conditions is questionable. The L, W, and LW are the most frequently used predictors due to their direct physical relation with LA and easiness to measure. Additionally, these basic predictors also exhibit consistency and accuracy in estimating LA. Most of LA estimation models adopt three regression types, i.e., linear, polynomials, and power regressions. Despite inherited weakness of standard linear and polynomial regression in estimating LA near the point of origin (Lakitan *et al.*, 2017). Up to now, few developed models were taken up the zero-intercept version of the linear and polynomial regressions.

Objective of this research was to find a pair of regression type and predictor trait for accurate and reliable leaf area estimation models for the habanero chili (*Capsicum chinense* Jacq.) cultivated at tropical lowlands.

Materials and methods

The research was conducted at the tropical lowland ecosystem in Indonesia during rainy season. The plants were grown in pots with dimension of 30 cm in diameter and 30 cm in height. Growing substrate consisted of soil-manure mixture at 3:1 based on volume (v/v). Seeds were soaked in tap water for 3 hours for separating viable and undesirable seeds. The floating seeds were casted-off and the good seeds were sown in the seedling treys. At age of 3 weeks, carefully chosen vigorous and relatively homogenous seedlings were transplanted to the prepared pots.

Leaves were collected at different sizes from the smallest unfolded young leaf to the largest available mature leaf for developing the leaf area (LA) estimation models. Larger leaves were collected from the main stem and smaller leaves were gathered at the branches. The leaves used for model development were intentionally picked on purposed to collect varied leaf sizes. The wide range and evenly distributed leaf sizes used determine legitimate extent of the developed models. Meanwhile, for model validation, the leaves were randomly picked.

The leaf of the habanero chili is single and has regular deltoid shape. Previous experience (Lakitan *et al.,* 2017, 2018, 2021; Meihana *et al.,* 2017; Widuri *et al.,* 2017) revealed that single leaf with regular shape does not require super large number of leaves to achieve high accuracy prediction, i.e., coefficient of determination (R^2) at 0.99 or higher, as long as wide range and even distribution of leaf size are assured. In this study, total of 318 leaves were used, consisted of 161 selected leaves for being used in model development and 157 random leaves were used for validation of the developed model.

Main predictors used are (1) leaf length (L), measured based on length of midrib from petiole-blade junction to tip of the leaf blade; (2) leaf width (W), measured at the widest part of leaf blade at direction perpendicular to the midrib; and (3) product of multiplication between leaf length and width (LW) which basically measuring an imaginary rectangular area with all of it four sides squarely around the leaf blade. The long side of the rectangular was parallel to direction of the leaf midrib. Leaf area, therefore, always be a proportional fraction of the rectangular area. Two additional predictors, i.e., L^2W and LW^2, are also used for exercising their effectiveness in estimating LA.

The trend of relationship between predictor used and estimated LA fits to linear, polynomial, and power regressions. If L or W was used as predictor, then the appropriate regressions to be used were second-order polynomial (also known as a quadratic regression) and power regression. The best fit for LW as predictor was the linear regression. The third-order polynomial regression fitted nicely with the double-bend curve created by L^2W or LW^2 as predictor. This study opted to use the zero-intercept version of the linear and polynomial regressions for a very fundamental reason, i.e., if the predictor was zero then the LA must also be zero. The zero-intercept regression is also known as the regression through the origin. By default, in power regression, LA was zero if the predictor was zero. Accuracy of each model was assessed based on the R^2 value.

The leaf area estimation models adopted formula of linear, polynomial, and power regressions. Other regressions, i.e., exponential and logarithmic, also compared to the selected three regressions. The developed models were validated using separated and randomly collected data. Accuracy was evaluated which based on calculated value of coefficient of determination (R^2).

Results

Assessing predictor and regression type

The type of regression is exercised for estimating area of leaf blade in the habanero chili using L, W, and LW as predictors (Table 1). If single predictor of L or W was used, the best fit models were polynomial and power regressions. Meanwhile, if LW was used, the best fit models were linear and power regression. Forcing the intercept to zero created better estimation for smaller leaves and did not significantly alter the coefficient of determination (R^2).

Table 1. Comparation amongst predictors and regression models on accuracy of leaf area estimation in habanero chili (*Capsicum chinense*)

Predictor	Regression type	Equation	R^2
Leaf length (L)	Linear	$LA = 7.7603L - 26.167$	0.9494
	Zero-intercept Linear	$LA = 5.2653L$	0.9381
	Quadratic	$LA = 0.2761L^2 + 2.693L - 7.2777$	0.9731
	Zero-intercept Quadratic	$LA = 0.3529L^2 + 1.0903L$	0.9709
	Exponential	$LA = 3.1415e^{0.2496L}$	0.8073
	Zero-intercept Exponential	$LA = e^{0.3588L}$	0.6447
	Logarithmic	$LA = 51.945 \ln(L) - 64.511$	0.7880
	Power	$LA = 0.5388L^{1.9307}$	0.9692
Leaf width (W)	Linear	$LA = 11.75W - 28.634$	0.9309
	Zero-intercept Linear	$LA = 7.7018W$	0.9266
	Quadratic	$LA = 0.709W^2 + 3.0377W - 6.6147$	0.9620
	Zero-intercept Quadratic	$LA = 0.8611W^2 + 0.9015W$	0.9603
	Exponential	$LA = 2.7783e^{0.3853W}$	0.7833
	Zero-intercept Exponential	$LA = e^{0.5297W}$	0.6346
	Logarithmic	$LA = 52.008 \ln(W) - 45.321$	0.7560
	Power	$LA = 1.028W^{1.9735}$	0.9592
Length x width (LW)	Linear	$LA = 0.64LW + 1.836$	0.9819
	Zero-intercept Linear	$LA = 0.6583LW$	0.9929
	Quadratic	$LA = -0.0008LW^2 + 0.7838LW - 1.8888$	0.9883
	Zero-intercept Quadratic	$LA = -0.0006LW^2 + 0.7402LW$	0.9876
	Exponential	$LA = 8.8002e^{0.0185LW}$	0.7438
	Zero-intercept Exponential	$LA = e^{0.0402LW}$	0.8268
	Logarithmic	$LA = 26.204 \ln(LW) - 55.722$	0.7784
	Power	$LA = 0.72LW^{0.9839}$	0.9962

Estimating LA using standard linear regression model and LW as predictor were satisfactory for most of the cases; however, it exhibited deviation within short range at lower end or near zero of the LW spectrum, i.e., in this case, if LW = 0 than LA = 1.836 cm^2. For instance, this deviation might be considered as negligible for leaf larger than 100 cm^2 but could be a problem for leaf smaller than 5 cm^2. For solving this issue, the zero-intercept linear regression could be used (Figure 1). Forcing LA = 0 if LW = 0 might also cause slight deviation but it should be negligible since it is spread across the LW spectrum. Changing from standard to the zero-intercept linear regression may lower the R^2, yet in this case, it improved the R^2 to 0.9929. All data deviates within $\pm$0.25 of the prediction line, i.e., between upper and lower broken lines.

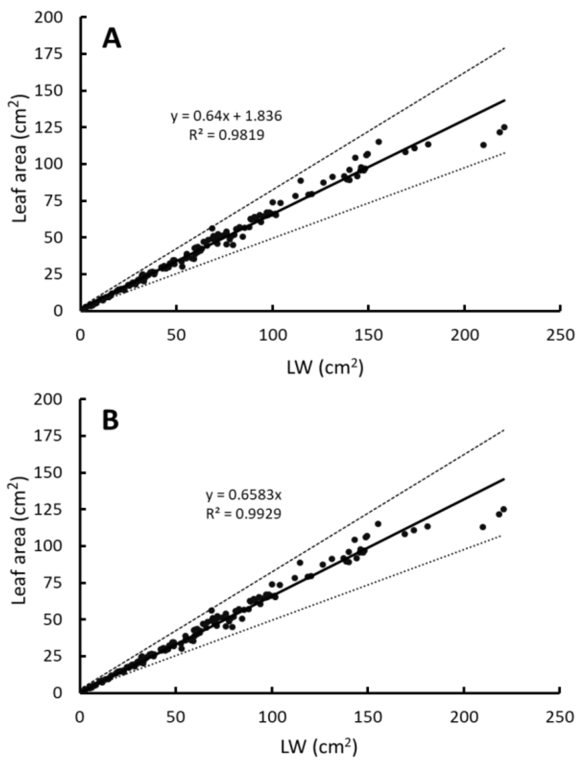

Figure 1. Comparison of standard (A) and zero intercept (B) linear regression models using LW as predictor

Data scattering pattern helped on recognizing its curving trend and should be used as reference in selecting an appropriate regression type. If single predictor of L or W was used, relationships between L and LA or W and LA were not linear, since process of leaf enlargement is not one direction. It is not solely due to increase of L or W independently, but also due to simultaneous two directional increases of L and W. Leaf growth is 3-dimentional in nature, including thickness. However, LA is only concern on area of leaf blade, therefore, it focuses only on L and W. Leaf volume is also dominated by L and W since leaf is very thin, except for succulent leaf. The leaf of habanero paper is non-succulent. Leaf blade thickness is less than 1 mm.

Application of the power regression in estimating LA using L or W is presented in Figure 2. The predicted line was nicely fit for both L and W with R^2 = 0.9881 and 0.9880, respectively. Use of the third-order polynomial regression in estimating LA was comparable to those of the power regression within range of L and W used in this study. However, extrapolation beyond this ranges for LA prediction should be cautiously inferred. By nature, the power regression will be continuously rising; meanwhile, the third-order polynomial regression will start to flatten at some points beyond these L and W ranges.

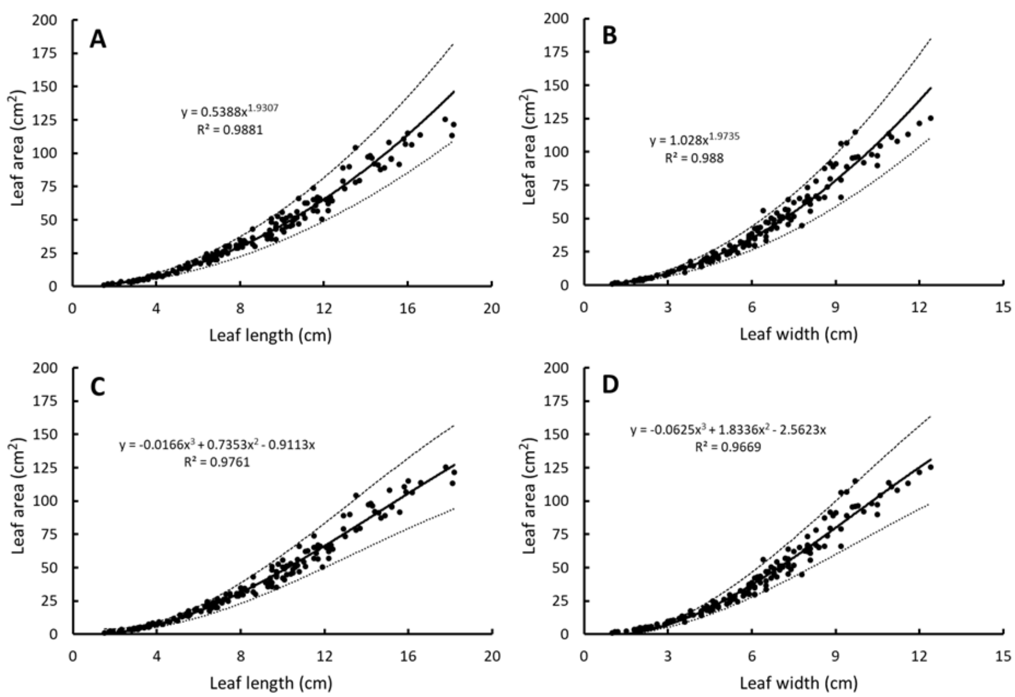

Figure 2. Selecting the regression types based on data distribution in leaf area estimation models developed using leaf length (A and C) and leaf width (B and D) as predictors and using power (A and B) and zero intercept third-order polynomial (C and D) regressions

Problem and solution associated with L^2W or LW^2 as predictor

Use of the second-order polynomial or quadratic regression for estimation of LA may encounter some inherited problem. For instances if L^2W or LW^2 are used as predictor. There is a weak argument of using these two predictors, but some do used them. The second-order polynomial regression curve has a single bend, therefore, far-end of the curve would either rise to infinity or curve down after a rising start. The latter case was exhibited in Figure 3.

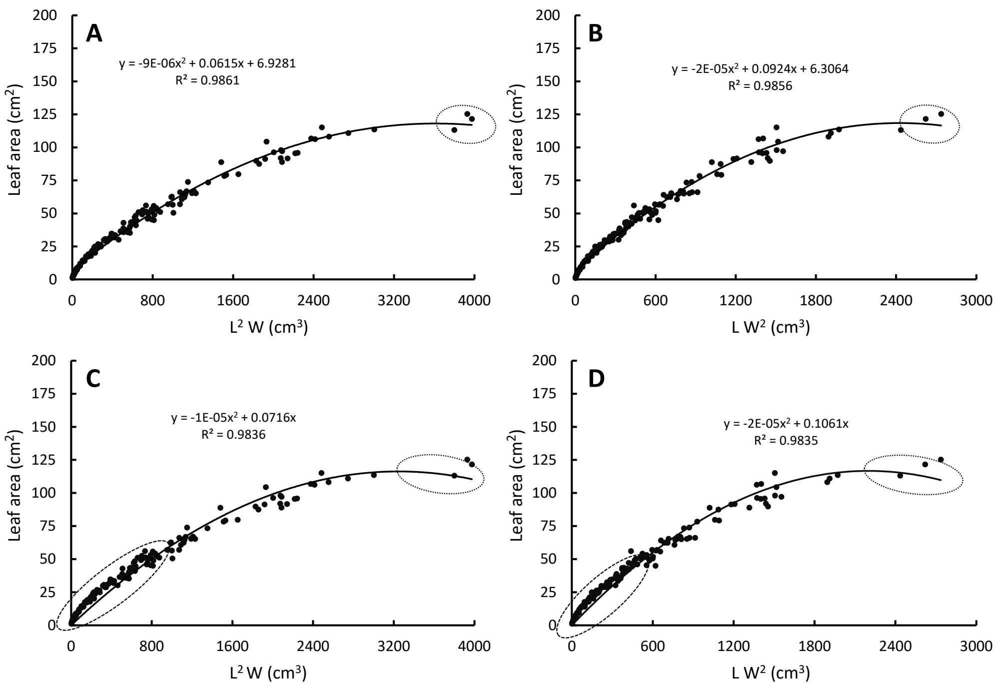

Figure 3. Some problems associated with using the standard (A and B) and zero-intercept (C and D) second-order polynomial regressions for estimation of leaf area using L^2W or LW^2 as predictor

The main problems were (1) the LA started to decrease as the value of predictor continue to increase beyond 3200 cm3 and 2400 cm3 for L^2W and LW^2, respectively; and (2) the estimated LA line was consistently fallen under the measured LA points for both L^2W and LW^2 if the zero-intercept second-order polynomial regression was applied (Figure 3C and 3D). Empirically, the application of standard and zero-intercept second-order polynomial models using L^2W and LW^2 as predictors resulted high R^2 values. In all cases, the R^2 value were higher than 0.98 (Figure 3A, 3B, 3C, and 3D). However, they inherited problems at both ends of the range of predictor used in developing the models.

A better LA estimation model for L^2W or LW^2 as predictor was the power regression (Figure 4). Both problems associated with the decrease of LA at higher predictor values and under-estimating of LA at lower predictor value were successfully eliminated by opting to use the power regression. As mentioned earlier, the second-order polynomial regression was well fitted at middle segment of collected L or W data (Figure 2). The encountered problems were not actually rooted on the selected regression type, but due to the use of inappropriate predictors. Therefore, in this study, validation of LA estimation models only uses L, W, and LW as predictors. L^2W and LW^2 as predictors were disregarded.

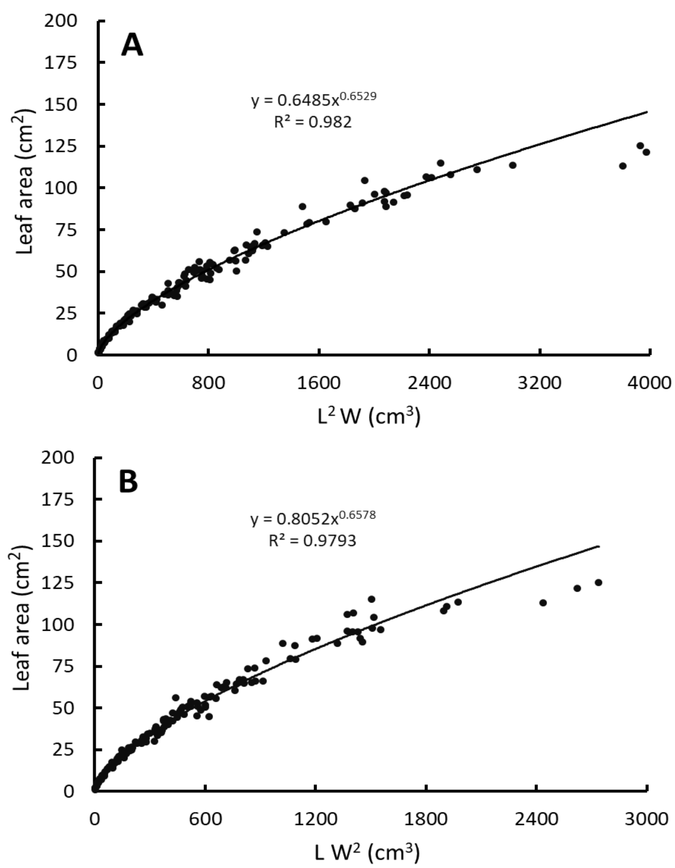

Figure 4. Power regression model provides good fit to data distribution using L^2W or LW^2 as predictors

Model validation

Different leaf samples were used in developing and validating the LA estimation models. Predicting LA using L tend to slightly under-estimate whereas using W tend to slightly over-estimate the measured value. Meanwhile, LA estimated using LW only deviated by 1.68 % of the directly measured LA (Figure 5). Area of LW is basically a rectangular of leaf length multiplied with leaf width and any measured leaf proportionally occupies part of the imaginary rectangle with length equals to L and width equals to W. Measurement of the L should be on or in direction of the leaf midrib, i.e., the straight line from leaf blade-petiole junction to tip of the leaf. The imaginary rectangle follows dimension of L and W of the measured LA. Since LA is always as a proportional fraction of LW and if LW = 0 then LA should also be zero; therefore, the most appropriate regression type for estimating LA is the zero-intercept linear model.

Based on high performance of the zero-intercept linear model using LW as predictor, validation of developed models was focused on the zero-intercept linear models using LW as predictor. However, the zero-intercept second-order polynomial model with L or W used as predictor was also validated as the options, if simpler or rapid data collection was required for some reasons. Accuracy and reliable of L or W as predictor was naturally slightly lower than if LW was used. The R^2 values were lower (0.9843 and 0.9899) for L and W than using LW (0.9960) in case of habanero chili (Figure 5).

Drawback in using weight-related traits as predictor

Specific leaf fresh weight (SLFW) and leaf water content (LWC) was significantly lower in smaller younger leaves with blade area less than 25 cm^2 (Figure 6). Significant differences in SLFW and LWC between smaller young leaves and larger mature leaves should be recognized as a source of discrepancy before considering using weight-related traits in developing LA estimation model. In addition, collecting data on weight-related traits in leaf is destructive in nature; therefore, it is not possible to do repetitive measurements over time on the same individual leaf. Instead, repetitive measurement can be achieved if dimension-related traits are used, which make it possible to calculate leaf expansion rate over any period on the same individual leaf.

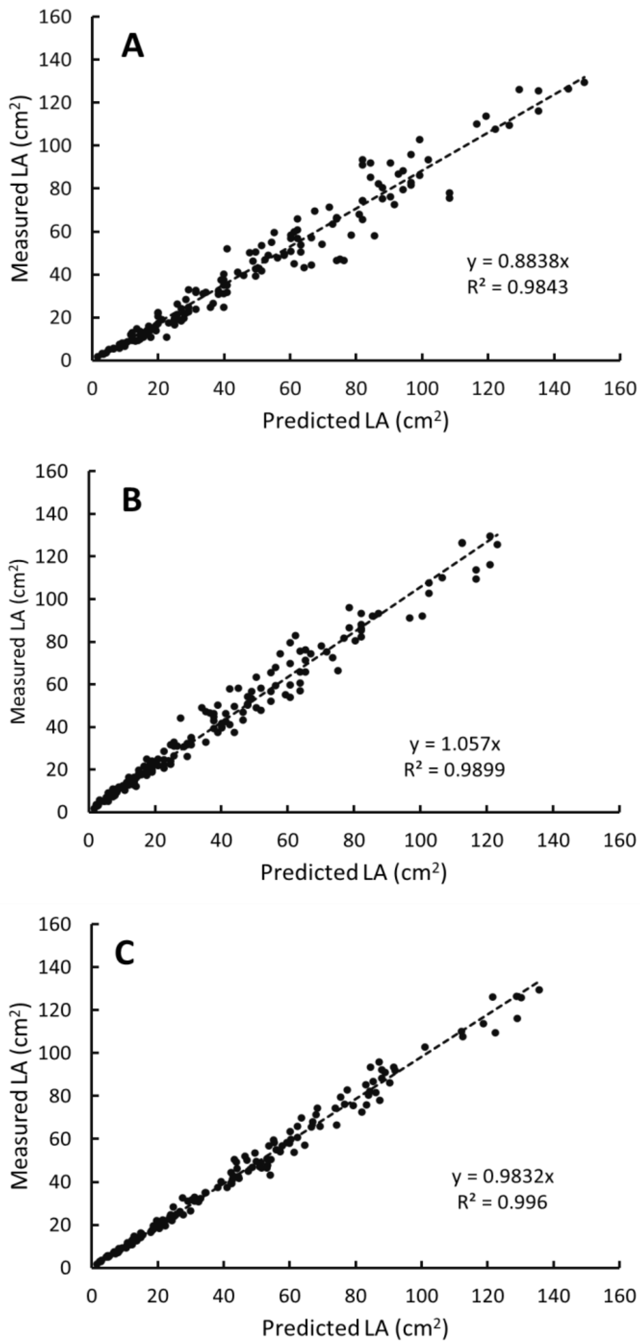

Figure 5. Validation of leaf area estimation models developed using leaf length (A), width (B), and LW (C) as predictors

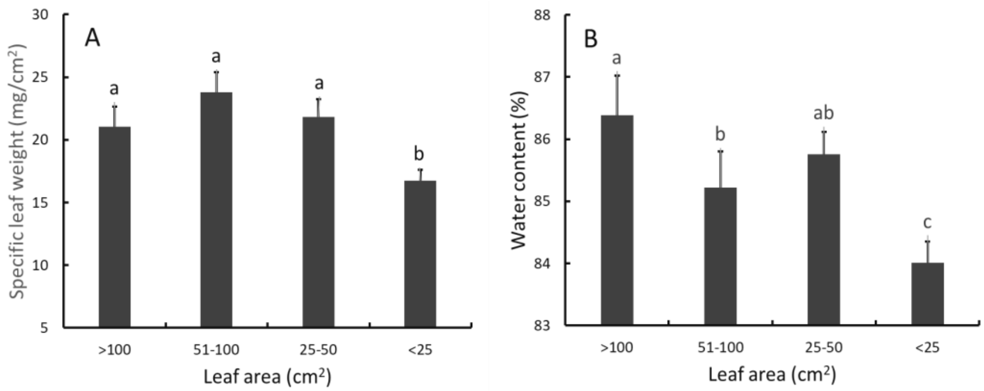

Figure 6. Specific leaf weight (A) and leaf water content (B) categorized based on leaf size in habanero chili

Steady L/W ratio indicated insignificant change in leaf shape as the leaf enlarge, starts from the leaf blade unfolded until the leaf had reached its maximum size. Figure 7 exhibited an overall stability of L/W ratio at around 1.5. However, higher variability of the L/W ratio was observed in smaller leaves or during early stage of leaf development. Variable leaf shape at early leaf development is common in most of plants. Nonetheless, the variability decreases as the leaf continue to grow approaching its mature size. The steady L/W ratio increased reliability of the LA estimation model.

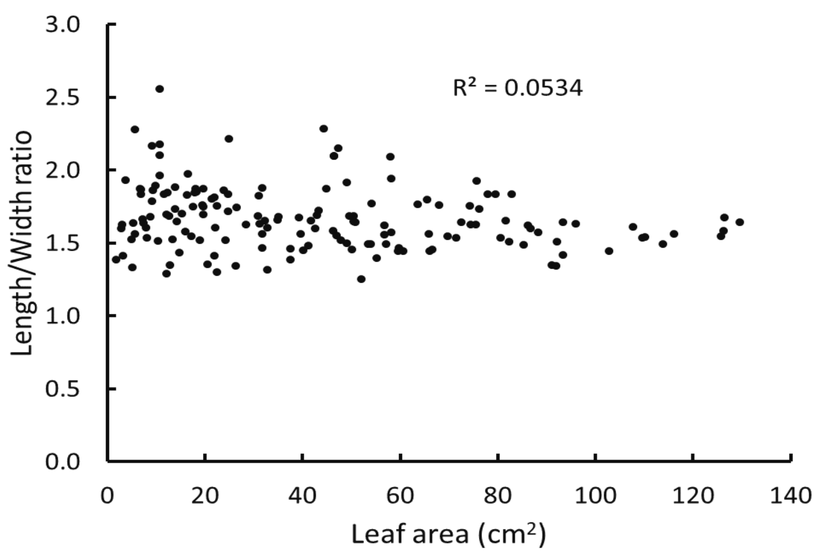

Figure 7. Despite high variability in smaller leaves, mean value of overall leaf length/width ratio was upheld steady as the leaf blade continues to expand

Optimum sample size for leaf area estimation model

Optimum sample size for LA estimation models are not received much attention. Sample size varied from hundreds to thousand leaves were observed in wide-range studies on this topics. Estimation of LA in single and regular leaf shape should require smaller leaf sample than for compound and/or irregular leaf shape. Use of 120 to 160 leaves were sufficient for creating an accurate LA estimation (Table 2) if the selected leaves were evenly distributed from the smallest to the largest available leaves in habanero chili.

Table 2. Optimum sample size for leaf area estimation in habanero chili

Predictor	Model	Leaf population size (%)			
		100	75	50	25
L	Power	$LA=0.539L^{1.931}$	$LA=0.560L^{1.918}$	$LA=0.530L^{1.939}$	$LA=0.528L^{1.935}$
	R^2	0.9692	0.9663	0.9691	0.9681
	R^2 drop	-	0.30	0.01	0.11
W	Power	$LA=1.028W^{1.935}$	$LA=0.999W^{1.987}$	$LA=1.031W^{1.972}$	$LA=1.011W^{1.984}$
	R^2	0.9592	0.9539	0.9445	0.9356
	R^2 drop	-	0.55	1.53	2.46
LW	Linear	$LA=0.6583LW$	$LA=0.6578LW$	$LA=0.6582LW$	$LA=0.6447LW$
	R^2	0.9929	0.9916	0.9912	0.9863
	R^2 drop	-	0.13	0.17	0.66
L^2W	Power	$LA=0.649(L^2W)^{0.653}$	$LA=0.658(L^2W)^{0.651}$	$LA=0.642(L^2W)^{0.655}$	$LA=0.635(L^2W)^{0.655}$
	R^2	0.9820	0.9794	0.9798	0.9721
	R^2 drop	-	0.26	0.22	1.01
LW^2	Power	$LA=0.805(LW^2)^{0.658}$	$LA=0.799(LW^2)^{0.659}$	$LA=0.803(LW^2)^{0.659}$	$LA=0.789(LW^2)^{0.661}$
	R^2	0.9793	0.9759	0.9722	0.9615
	R^2 drop	-	0.35	0.71	1.82
Number of leaf		160	120	80	40

L and LW were more reliable amongst compared predictors in this study, with the R^2 drop was less than 1% even after leaf sample size was reduced to 25%. Meanwhile, the R^2 dropped more than 1% on other predictors if the sample size was reduced to 25%. The drops were 1.01% and 1.82% for L^2W and LW^2, respectively. The largest R^2 drop of 2.46% was occurred if W was used as predictor which was consistent with the fact that W was the weakest predictor (R^2 = 0.9592) for LA in habanero chili. This also indicated that high variability of LA was observed at different value of W.

Discussion

Application of multiple predictors

Allometric models for non-destructive leaf area estimation has been developed based on measurements on morpho-physical traits and multiplication of the traits. They were directly related to dimension of the leaf blade, i.e., L or W; multiplication of these basic dimensions, including LW, LL, WW, L^2W, LW^2, L/W, or W/L. Other traits may also be used, including petiole length, leaf thickness, and leaf fresh weight. However, the most frequently used predictor in leaf area estimation model was LW which had been used in many different plants, including in walnut (Keramatlou *et al.*, 2015), durian (Sankar *et al.*, 2017), papaya (Oliveira *et al*., 2019), and bell pepper (Padrón *et al*., 2016).

Very few LA estimation models did not include LW as predictor (Ghoreishi *et al*., 2012; Giaccone *et al*., 2017). For simplicity reason, some only used L and/or W as predictor. However, almost in all cases, LW as predictor was more accurate than either L or W separately (Koubouris *et al*., 2018). Furthermore, Pompelli *et al.* (2012) added that if either L or W alone was used, besides the accuracy became lower compared to the use of LW, it also increased heteroscedastic residual dispersion. This is true in the most cases (Lakitan *et al*., 2017; 2018; 2021).

Mack *et al*. (2017) developed LA estimation model with two predictors (LL and LW) using multiple linear regression of LA = $\alpha \times \beta LL \times \gamma LW$ and claimed that this model as the most accurate in their study on chia plant (Salvia hispanica); yet they also acknowledged that using LW as predictor was also as accurate. Ghadami-Firouzabadi *et al*. (2015) used $LW_{0.5}$ instead of LW for better estimating LA in sunflower plant using the exponential model. Hinnah *et al.* (2014) also used L^2W and LW^2 as predictors but concluded that used of LW with power regression was the best option as indicated by the highest R^2. This concludes that effort to build up more predictors might result in increases of the R^2 for a specific case, but the results cannot be generalized. In more diverse cases, use of the simple LW as predictor exhibited consistent performance.

Advantage of zero-intercept regression model

Most frequently use models for LA estimation with LW as predictor was the standard linear regression of LA = $\alpha + \beta LW$ (Keramatlou *et al*., 2015: Sankar *et al.*, 2017: Oliveira *et al.*, 2019; Padrón *et al*., 2016). Symbol α is for the intercept and β is the slope. Use of the standard linear model assures the LA accuracy within range of LW used in developing the model. Potential problem may occur in extrapolating the model to cover smaller leaf outside the range. Theoretically, in standard linear regression, if L = 0 or W = 0, but most likely the LW is not zero, which is unrealistic. Some models use the zero-intercept linear regression for overcoming the problem associated with the standard linear model. Accuracy of the zero-intercept linear regression model had been proven in *Crotalaria juncea* (Carvalho *et al*., 2017), trifoliate leaf of snap bean (Lakitan *et al.*, 2017), tomato compound leaf (Meihana *et al.*, 2017), chili pepper (Widuri *et al*., 2017), celery compound leaf (Lakitan *et al*., 2021), and yellow velvetleaf plant (Lakitan *et al*., 2018).

The linear model should not be used if L or W is separately used as predictor since increase in L is not always proportional to increase in W during leaf enlargement process. The L/W ratio could vary among individual leaf even if the leaves are collected from single individual plant. Appropriately used models for L or W as predictor are the power regression (Pompelli *et al*., 2012) and the zero-intercept second-order polynomial regression. Both regressions retain LA = 0 if either L or W = 0.

Standard second and third order polynomial had been used by Eftekhari *et al*. (2011) in grape leaf, Mazzini *et al.* (2010) in citrus leaf, Salazar *et al.* (2018) in cacao leaf, Pezzini *et al*. (2018) in pigeon pea, and Basak *et al.* (2019) in pepper. The weakness of standard linear and polynomial models is if L = 0 or W = 0 then most likely LA > 0 or LA < 0 and less likely LA = 0. Some researchers were aware of this issue and opted to use the power regression (Pompelli *et al.*, 2012; Pezzini *et al.*, 2018) or the zero-intercept linearor the zero-intercept polynomial regressions (Lakitan *et al.*, 2017, 2018, 2021; Meihana *et al*., 2017; Widuri *et al.*, 2017; Carvalho *et al*., 2017).

Dimention-based versus Weight-based predictors

SLFW commonly associates with leaf thickness and leaf water content. SLFW was affected by temperature (Jumrani *et al*., 2017), plant phenology (González-Pérez, 2018), and drought stress (Zhang *et al*., 2015) but did not affect by shallow soil water table (Meihana *et al.*, 2017). LWC is affected at both ends of soil-plant water continuum, i.e., root water uptake and transpiration rate. In turn, dynamics of LWC will affect SLFW. Differences in SLFW and LWC between smaller young leaves and larger mature leaves were disclosed in this study (Table 6). Uncontrollable effects of external (climatic and soil conditions) and internal (whole-plant water status) on leaf growth possess a potential discrepancy which directly associated with weight-based predictors. Meanwhile, dimension-based predictors (L, W, and their multiplications) do not have these discrepancy issues since these predictors are directly related to LA. Both L and W are the post-effect of the external conditions.

On average, the L/W ratio was relatively unchanged during leaf growth and development in habanero chili. Stability of the L/W value is frequently used as indicator of unchanged leaf shape during leaf growth (Keramatlou *et al.*, 2015). While Shi *et al.* (2019) used the LA/L ratio for detecting unchanged shape of the examined leaf. Stability of the L/W ratio or LA/L ratio increased reliability of the LA estimation model.

Optimal leaf sample size

Number of leaves used in developing or validating LA estimation models was wide-ranging from hundreds to thousands. The optimal number should be associated with diverse shape of the leaf, from simple, regular, and perfectly flat single leaf type to complicated, randomly shape, and wrinkled compound leaf type. Cargnelutti-Filho *et al*. (2015) proved that use of 200 leaves were sufficient for constructing an accurate power model for the LA in jack beans. Others used between 300-499 leaves for faba bean (Peksen, 2007), grave (Tsialtas *et al.,* 2008), and *Jatropha curcas* (Pompelli *et al.*, 2012); used between 500-1000 leaves for sunflower (Rouphael *et al*., 2007) and squash (Toebe *et al.,* 2019); and more than 1000 leaves for grape (Buttaro *et al*., 2015). Result of this study indicated that used of around 100-200 leaves was adequate for simple, regular, and flat single leaf in habanero chili. The accuracy in LA estimation was proven by the $R^2 > 0.99$. This was achieved if the selected leaves were widely varied in size yet evenly distributed.

Based on results of this research, it is recommended to use the zero-intercept linear, zero-intercept polynomial, or power regression for developing accurate, technically simple, reliable, and geometrically-sound LA estimation models. Use of LW as predictor suitably matches with the zero-intercept linear model; L or W is compatible with the zero-intercept second-order polynomial model; meanwhile, the power regression model is fit well with all L, W, and LW. Differences in SLFW and LWC between smaller and larger leaves indicated that leaf weight is varied in each unit of leaf area and the weight per unit area is also fluctuated due to dynamics of LWC. Therefore, weight-based traits are not appropriate predictors for estimating LA. The steady L/W ratio indicated that the leaf shape is relatively unchanging during leaf growth. This finding confirms that the zero-intercept linear model with LW as predictor is an accurate and reliable for LA estimation for the habanero chili (LA = 0.6583LW; $R^2 = 0.9929$). Sample size between 120 to 160 leaves is sufficient for achieving accurate LA estimation in habanero chili if wide range in size and evenly distributed within the leaf sample is attained. This finding has potential for being used in all species of Capsicum, but validation procedures have to be fulfilled.

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