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Short Communication: Allometric model to estimate bifoliate leaf area and weight of kaffir lime (*Citrus hystrix*)

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Abstract. *Budiarto R, Poerwanto R, Santosa E, Efendi D, Agusta A. 2021. Short Communication: Allometric model to estimate bifoliate leaf area and weight of kaffir lime* (Citrus hystrix). *Biodiversitas 22: 2815-2820.* Leaf is an economically important plant organ harvested from kaffir lime (*Citrus hystrix* DC.) for flavor and fragrance. This study aimed to formulate and validate regression models to estimate the leaf area (LA) and leaf weight (LW) of bifoliate kaffir lime leaf. There were 220 bifoliate leaves collected from 22 individual plants planted on Bogor. Bifoliate *C. hystrix* leaf consisted of upper main leaflets and winged petiole as lower secondary leaflet. All leaves were pooled and then grouped randomly into two subgroups for model formulation and validation, respectively. Linear, zero intercepts linear, exponential, logarithmic, polynomial, zero intercept polynomial and power regressions were used to properly estimate LA and LW. There was 63 formula obtained from nine predictors following seven regression models. Selected nine formulas with the highest R^2 plus a stepwise formula were further tested for validation. Stepwise always showed the highest R^2 followed by total of leaf ,length (TL) both on LA and LW estimation. However, the stepwise seemed to be more complicated and time wasted than TL regression model. Thus, our recommendation models for non-destructive and simple estimation in *C. hystrix* bifoliate leaf were LA = 0.1997 $(TL)^2$ + 0.4571 (TL) and LW = 0.0067 $(TL)^2$ + 0.0065 (TL), respectively.

Keywords: Leaf length, non-destructive measurement, regression, stepwise, zero intercept polynomial

INTRODUCTION

Kaffir lime (*Citrus hystrix* DC.) is minor citrus with commercial values on its leaves that used for flavor or fragrance worldwide (Wongpornchai 2012; Mabberley 2004). In agribusiness of kaffir lime, the leaf area and leaf weight are critical factors that influenced the final yield and farmers income (Budiarto et al. 2019a). Many scientists used leaf measurement in various studies of ecology, plant stress, plant-environment interaction, and precision agriculture (Sestak et al. 1971; Kinhal 2019). In earlier reports, both leaf variables also good proxies of plant growth (Salazar et al. 2018) and plant physiological condition (Bleasdale 1984) in response to various stressing condition, such as pruning (Palliotti and Poni 2011; Budiarto et al. 2018), grafting (Blanco and Folegatti 2005), shading (Budiarto et al. 2019b), interspecific plant competition (Harper 1977), elevated CO_2 (Ewert 2004), drought (Shekafandeh and Hojati 2012; Adamipour et al. 2016; Dadashpour et al. 2017), and salinity (Qrunfleh et al. 2017). Leaf area is frequently used to monitor the biomass accumulation (Potter and Jones 1977; Weraduwage et al. 2015), leaf expansion, and also net assimilation rate (Lakitan et al. 2017), while leaf weight is used to measure relative growth rate, leaf harvesting index (Salazar et al. 2018), and biomass accumulation (Tieszen 1982).

Both leaf area and leaf weight can be measured by destructive and non-destructive methods. The destructive method is not suitable for time series observation, while non-destructive method allows the leaf to exposed by repeated observation (De Swart 2004). Non-destructive method can be performed based on mechanical instruments such as laser scanner and leaf area meter; however, it is not easily accessible everywhere due to its sophisticated character and highly depend on the electrical source for operation (Huang and Pretzsch 2010; Lakitan et al. 2017). Non-destructive method can also be performed by allometric approach that seems to be more reliable and feasible on limited budget and condition because of the simple tool required. Numerous studies have reported the success of allometric for estimating plant biomass (Karyati et al. 2019, 2021; Wirabuana et al. 2020).

However, the weakness of allometric approach is limited to specific plant genotypes, due to the genetic variability for such leaf allometric characters, so that further studies for each genotype are required (Malagi et al. 2010). Allometric approaches to estimate leaf area are widely developed in various types of plants, such as *Actinidia deliciosa* (Mendoza-de 2007), *Mangifera indica* (Ghoreishi et al. 2012), *Diospyros kaki* (Cristofori et al. 2008), and others. Moreover, the leaf area model for single and trifoliate leaf of several citrus genotypes has already developed (Mazzini et al. 2010; Dutra et al. 2017), excluding the kaffir lime. Kaffir lime leaf exhibit bifoliate character instead of single or trifoliate ones, indicated by the presence of winged petiole alike secondary leaflet below the main one (Budiarto et al. 2021). There is a lack of studies regarding leaf area and leaf weight prediction model specialized for bifoliate leaf of kaffir lime. Therefore, this study aimed to formulate and validate non-destructive simple regression models to estimate the area and weight of bifoliate *C. hystrix* leaf.

MATERIALS AND METHODS

Measured samples were 220 kaffir lime leaves harvested from 22 kaffir lime seedlings on December 2017 at Pasir Kuda experimental farm of IPB University, Bogor, Indonesia (263 m a.s.l.; 6°36’32.6” S, 106°47’0.9” E). The basic criteria for leaf selection were no malformation, disease-free and fully developed leaf. This method accommodated the smallest up to the largest leaf size available in the stock plants. This approach was similar to previous studies, such Lakitan et al. (2017) and Tondjo et al. (2015).

Data collection and analysis

Each selected leaf was coded and directly transferred one by one to analytical balance to obtain the actual fresh weight (Wr). Later on, the coded leaf was scanned by electronic scanner-printer to obtain the real leaf area (Ar) of each sample. Leaf area was calculated on the scanned results by using image analysis software, namely ImageJ (Schneider et al. 2012) version 1.50. The scanned document was also used to measure several predictors, i.e the upper leaflet width (UW), the lower leaflet width (LW), the upper leaflet length (UL), the lower leaflet length (LL), the total of leaf ,length (TL = UL + LL), the imaginary rectangular leaf area of upper leaflet (UR = UW × UL), the imaginary rectangular leaf area of lower leaflet (LR = LW × LL), the imaginary elliptical leaf area of upper leaflet (UE = 3.14 × 0.25 × UW × UL), the imaginary elliptical leaf area of lower leaflet (LE = 3.14 × 0.25 × UW × UL) and the stepwise (S). Predictors were grouped as simple, double, and multiple variables. The simple predictors were derived from the width and length of upper and lower leaflets such as UW, LW, UL, LL. The double predictors were derived from the imaginary combination of two simple predictors in form of the elliptical and rectangular leaf area such as UR, LR, UE, LE. The multiple predictors are stepwise that was combination of four simple predictors, i.e. UW, LW, UL, and LL. The illustration of the measurement of the length and width in the bifoliate kaffir lime leaf was depicted in Figure 1.

The allometric data of leaves were pooled and equally divided into two subgroups, for model formulation and validation. There were 110 leaves used for model formulation, so do the validation. The size of data used both for model formulation and also validation was clearly described in Table 1.

Regression analysis was performed in Microsoft Excel 2016 to show the relationship of predictors to Ar and Wr from the model formulation subgroup. Seven regression analyses were used for every predictor dataset namely linear, zero intercepts linear, exponential, logarithmic, polynomial, zero intercept polynomial, and power regressions. Every single regression analysis produced the coefficient of determination (R^2) that used to evaluate the regression appropriateness. The higher R^2, the closer the data to the produced regression lines meant the better regression, and *vice versa* (Rawlings et al. 1998). The highest R^2 for every predictor was selected and that regression was nominated as the potential model. To determine the proper model among potential nominees, the validation test is launched. To validate, Pearson’s correlation analysis was performed to test the strength and direction of the relationship between the estimated and the actual measurement of LA and LW from the model formulation subgroup. The model with the strongest correlation coefficient value and the simplest predictor required was selected as the recommendation.

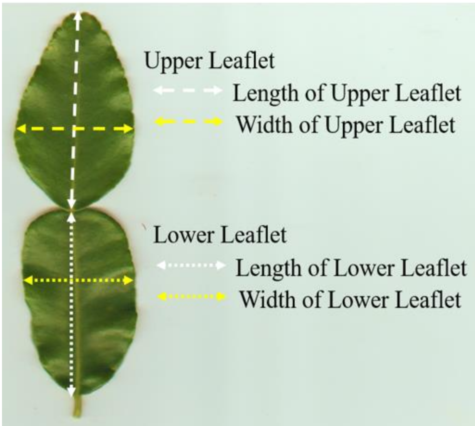

Figure 1. Illustration of the measurement of the length and width in the bifoliate leaf of kaffir lime

Table 1. Data size for model formulation and validation of kaffir lime (*Citrus hystrix*) leaf weight and leaf area

Value	Model formulation		Model validation	
	Leaf area (cm^2)	Leaf weight (g)	Leaf area (cm^2)	Leaf weight (g)
Minimum	7.24	0.19	3.81	0.10
Maximum	39.52	1.28	26.57	0.83
Mean	22.62	0.67	15.00	0.44
Median	21.26	0.60	15.17	0.43
Count	110	110	110	110
SD	8.05	0.26	5.48	0.17

Note: Count: The number of data, SD: Standard deviation for data

RESULTS AND DISCUSSION

Regression analysis is defined as powerful method in statistical modeling to show the relationship between two or more variables so that one variable can be predicted from other variables (Rawlings et al. 1998). Previous studies used regression analysis to estimate citrus chlorophyll content (Barman and Choudhury 2020), citrus maturity level (Itakura et al. 2019), and assessment of citrus chemical quality variables (Torres et al. 2019). Present work used regression analysis to statistically estimate the leaf area and leaf weight of kaffir lime by observing some predictors. The R^2 of seven regression models from nine predictors for estimating leaf area and leaf weight of kaffir lime was displayed in Table 2. In most predictors such as UW, LW, LL, UR, LR, UE, LE, the highest R^2 derived from power regression model, while UL and TL showed the polynomial and zero intercept polynomial regression model as the highest ones. Power and polynomial model proved to be more powerful than linear, exponential, and logarithmic. Besides being lower, the use of linear regression was considered less realistic, because the mathematical equation of the linear regression contained intercept value. When the equation had intercepted, the prediction could not apply to the zero value or below. The intercept could be forced to be zero, however the zero intercept linear was also unproper to use because of the considerable drop of R^2.

The different thing happened in terms of polynomial regression. This study used the second level of polynomial regression, also called quadratic regression. In some predictors, the highest R^2 showed by polynomial regression than the zero intercept ones. However, the use of polynomial regression was less realistic than the zero intercept ones. This work preferred to recommend the zero intercept polynomial regression because there was only slightly drop of the R^2 produced by the zero intercept polynomial regression compared to the normal ones, unlike the linear and its zero intercept case.

In general, the mathematical equation showed in regression graph could be composed of varied variables depend upon the type of regression models. The details of mathematical equation of the selected regression model (the highest R^2) from every predictor were showed in Table 3. For the stepwise regression model, the mathematical equation composed of the dependent variable (LA or LW), the explanatory variables (UL, LL, UW, LW), the slope (value in front of the explanatory variables), and the minus intercept value. For the zero intercept polynomial regression model, the mathematical equation composed of the dependent variable (LA or LW), the explanatory variables (predictors), the slope (value in front of the explanatory variables) with the quadratic function. For the power regression model, the mathematical equation composed of the dependent variable, the explanatory variables, and the slope, with no intercept and formed power function. Those equations were collected and subsequently used in validation step. The predictions of leaf area and leaf weight were made by measuring the predictors from the validation subgroup and then calculating those equations.

Table 2. Coefficient determination values (R^2) of seven regression models from nine predictors for estimating leaf area and leaf weight of kaffir lime (*Citrus hystrix*)

Regression model	Predictors								
	UW	LW	UL	LL	TL	UR	LR	UE	LE
Leaf area estimation									
Linear	0.83	0.83	0.82	0.72	0.88	0.88	0.86	0.88	0.86
Zero Intercept Linear	0.7	0.74	0.66	0.69	0.72	0.88	0.81	0.88	0.81
Exponential	0.83	0.83	0.82	0.73	0.88	0.85	0.82	0.85	0.82
Logarithmic	0.82	0.8	0.79	0.71	0.85	0.86	0.84	0.86	0.84
Polynomial	0.83	0.83	0.83	0.73	0.89	0.89	0.88	0.89	0.88
Zero Intercept Polynomial	0.82	0.83	0.83	0.73	0.89	0.88	0.88	0.88	0.88
Power	0.85	0.84	0.82	0.74	0.88	0.9	0.88	0.9	0.88
Leaf weight estimation									
Linear	0.83	0.82	0.77	0.65	0.8	0.86	0.81	0.86	0.81
Zero Intercept Linear	0.67	0.7	0.6	0.6	0.64	0.86	0.79	0.86	0.79
Exponential	0.85	0.83	0.78	0.65	0.81	0.84	0.78	0.84	0.78
Logarithmic	0.82	0.78	0.73	0.63	0.77	0.84	0.78	0.84	0.78
Polynomial	0.83	0.82	0.78	0.65	0.82	0.86	0.82	0.86	0.82
Zero Intercept Polynomial	0.83	0.82	0.78	0.65	0.81	0.86	0.82	0.86	0.82
Power	0.87	0.84	0.78	0.66	0.81	0.89	0.83	0.89	0.83

Note: UW: upper leaflet width, LW: lower leaflet width, UL: upper leaflet length, LL: lower leaflet length, TL: total of leaf length, UR: imaginary rectangular leaf area of upper leaflet, LR: imaginary rectangular leaf area or lower leaflet, UE: imaginary elliptical leaf area of upper leaflet, LE: imaginary elliptical leaf area of lower leaflet.

Table 3. Mathematical equation of selected regression model from every predictor for estimating leaf area and leaf weight of kaffir lime (*Citrus hystrix*)

Predictors	Regression model	Mathematical equation	R^2
Leaf area estimation			
S	Stepwise	LA = 1.93 (UL) + 2.10 (LL) + 4.30 (UW) + 3.71 (LW) - 20.68	0.975
UW	Power	$LA = 2.702\ (UW)^{1.7605}$	0.851
LW	Power	$LA = 4.5564\ (LW)^{1.5435}$	0.837
UL	Zero Intercept Polynomial	$LA = 0.7497\ (UL)^2 + 0.6968(UL)$	0.826
LL	Power	$LA = 2.8408\ (LL)^{1.3726}$	0.741
TL	Zero Intercept Polynomial	$LA = 0.1997\ (TL)^2 + 0.4571\ (TL)$	0.888
UR	Power	$LA = 1.4803\ (UR)^{0.9649}$	0.901
LR	Power	$LA = 2.8787\ (LR)^{0.8116}$	0.878
UE	Power	$LA = 1.8681\ (UE)^{0.9649}$	0.901
LE	Power	$LA = 3.5011\ (LE)^{0.8116}$	0.878
Leaf weight estimation			
S	Stepwise	LW = 0.0458 (UL) + 0.0441 (LL) + 0.1685 (UW) + 0.1466 (LW) - 0.7096	0.943
UW	Power	$LW = 0.0645\ (UW)^{1.938}$	0.868
LW	Power	$LW = 0.1159\ (LW)^{1.6882}$	0.843
UL	Zero Intercept Polynomial	$LW = 0.0258\ (UL)^2 + 0.0027\ (UL)$	0.78
LL	Power	$LW = 0.0785\ (LL)^{1.4145}$	0.663
TL	Zero Intercept Polynomial	$LW = 0.0067\ (TL)^2 + 0.0065\ (TL)$	0.814
UR	Power	$LW = 0.0351\ (UR)^{1.0425}$	0.885
LR	Power	$LW = 0.0747\ (LR)^{0.8621}$	0.834
UE	Power	$LW = 0.0451\ (UE)^{1.0425}$	0.885
LE	Power	$LW = 0.0919\ (LE)^{0.8621}$	0.834

Note: S: stepwise, UW: upper leaflet width, LW: lower leaflet width, UL: upper leaflet length, LL: lower leaflet length, TL: total of leaf length, UR: imaginary rectangular leaf area of upper leaflet, LR: imaginary rectangular leaf area or lower leaflet, UE: imaginary elliptical leaf area of upper leaflet, LE: imaginary elliptical leaf area of lower leaflet.

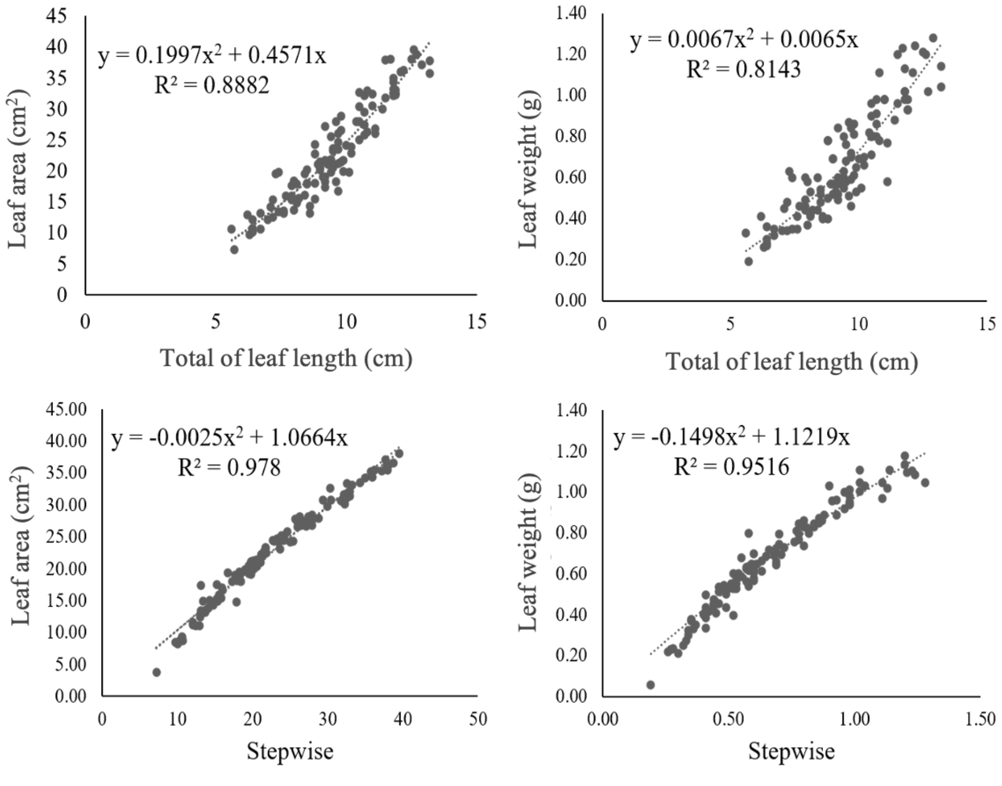

Figure 2. Scatter plot regression of two recommended models derived from total of leaf length and stepwise for estimating leaf area and leaf weight of kaffir lime (*Citrus hystrix*)

Table 4. Pearson's correlation coefficient of 10 predictors between data sets of the estimated and the actual measurement of leaf area and leaf weight of kaffir lime (*Citrus hystrix*)

Leaf variables	**Predictors**									
	S	**UW**	**LW**	**UL**	**LL**	**TL**	**UR**	**LR**	**UE**	**LE**
Leaf area	0.97	0.92	0.84	0.88	0.86	0.93	0.93	0.91	0.93	0.91
Leaf weight	0.83	0.77	0.74	0.74	0.77	0.81	0.78	0.81	0.78	0.81

Note: S: stepwise, UW: upper leaflet width, LW: lower leaflet width, UL: upper leaflet length, LL: lower leaflet length, TL: total of leaf length, UR: imaginary rectangular leaf area of upper leaflet, LR: imaginary rectangular leaf area or lower leaflet, UE: imaginary elliptical leaf area of upper leaflet, LE: imaginary elliptical leaf area of lower leaflet

Correlation analysis was widely used statistical tool to measure the direction and strength of certain relationships between two correlated components (Han and Kamber 2006). The relationship is numerically determined by decimal value, called as the correlation coefficient. In general, the correlation coefficient ranged from -1 up to 1, which meant a strong negative relationship for -1, no relationship at all for 0, and a strong positive relationship for 1. Strong relationship meant the independent variable can be strongly predicted the dependent variable (Kumar and Chong 2018). Previous studies used correlation approach to reveal the relationship between (i) citrus rind quality and nutrient content (Khalid et al. 2012), (ii) leaf nutrient content and pummelo citrus fruit production (Thamrin et al. 2014), (iii) pummelo fruit-producing characters and fruit number per plant (Hossain et al. 2018), and (iv) lemon fruit chemical content and its antioxidant activity (Dong et al. 2019). Present work used correlation analysis to validate the formulated allometric model. The data sets of the estimated leaf area and leaf weight were correlated to the Ar and Wr of the validation subgroup. Our data possessed positive coefficient of correlation ranged from 0.84 up to 0.97 for LA and 0.74 up to 0.83 for LW (Table 4). The highest correlation coefficient was found in stepwise for the multiple predictors and the TL for the single predictor, both for leaf area and leaf weight estimation.

Two potential model nominees were stepwise regression model and zero intercept polynomial regression model of total of leaf length, either for LA and LW estimation (Figure 2). Stepwise regression showed stronger relationship than zero intercept polynomial due to the more predictors involved. The mathematical equation of stepwise regression required four predictors to help the estimation. In each step of stepwise regression, a variable is considered for addition to or subtraction from the set of explanatory variables. However, it was more complicated to perform stepwise regression model due to the more predictors needed, compared to the simple predictor of total of leaf ,length with its zero intercept polynomial regression model.

In short, for the simple, non-destructive and unsophisticated kaffir lime leaf area and leaf weight estimation, this study recommended the use of the simple predictors of total of leaf ,length with zero intercept polynomial regression model. The equation to estimate leaf area and leaf weight were LA = 0.1997 $(TL)^2$ + 0.4571 (TL) and LW = 0.0067 $(TL)^2$ + 0.0065 (TL), respectively. In formulation step, the coefficient of determination of those models were 0.8882 for LA estimation and 0.8143 for LW estimation. In validation step, the coefficient of correlation of those models was 0.933 for LA estimation and 0.805 for LW estimation.

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