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Simplifying procedure for a non-destructive, inexpensive, yet accurate trifoliate leaf area estimation in snap bean (*Phaseolus vulgaris*)

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Abstract

Non-destructive measurement of leaf area (LA) is preferred in growth analysis and plant physiological studies. Many regression-based models have been developed for estimating LA using leaf length (L), leaf width (W), or imaginary rectangle of L x W (LW) as predictor or independent variable. Objective of this study was to develop and validate appropriate regression models for estimating snap bean trifoliate LA using easily measured L, W, or calculated LW. Snap bean used in this research was PV072 cultivar. Trifoliate-leaf samples were purposively collected from different individual plants, to represent wide range of leaf sizes, from the smallest leaf with fully open blade to the largest available leaf. Snap bean trifoliate leaf consists of three leaflets. The sampled leaves were alternately divided into two subgroups, based on length of terminal leaflet, for developing and validating LA estimation models. Linear, quadratic, and power regressions were evaluated for their appropriateness to be used for estimating LA. Intercept (α) was forced to zero to make the models more geometrically realistic. Results of this research indicated that: (1) zero-intercept quadratic and power regression models performed well for length of leaflet (Lt) or width of leaflet (Wt) was used as predictor, whereas zero-intercept linear model was appropriate and geometrically-sound if imaginary rectangular Lt x Wt (LtWt) was used for estimating surface area of both terminal and side leaflets (LtA); (2) for a practical, fast, and accurate estimation of LA, LtWt of terminal leaflet was the recommended option among other single or combination of predictors; and (3) recommended empirical model for LA estimation of snap bean trifoliate leaf is LA = 1.5198 LtWt.

Key words: trifoliate leaf, leaflet, estimation model, growth analysis, leaf expansion

Introduction

Leaf area (LA) is a frequently used parameter in plant growth analysis and plant physiology studies. LA is used as based for calculating net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA), and leaf area index (LAI); measuring photosynthetic, respiration, and transpiration rates; and monitoring leaf expansion rate (LER) during leaf development or in response to environmental stress conditions, especially due to nutrient and water deficiency.

Jordan-Meille and Pellerin (2004) found that K deficiency reduced leaf elongation rate (LeR) and believed that these results strengthen the idea that leaf growth is a key variable for analyzing, and later on modeling, crop growth under K deficiency. Similarly, Plenet *et al*. (2000) reported that LeR and final size of leaves were significantly reduced but leaf elongation duration was not greatly affected by P deficiency. Leaf area at harvest was also significantly reduced due to nitrogen deficiency (Zhao *et al*., 2003).

Water stress affected leaf expansion and translocation of assimilates from leaf to stem, and resulted in thicker leaves and lower SLA (Zhang *et al*., 2015). Moreover, Sellin *et al*. (2012) found that there was size-dependent variability of leaf and shoot hydraulic conductance. The bigger the leaf area the higher hydraulic conductance. Leaf anatomical study of *Betula pendula* saplings revealed that this trend was attributable to enhanced vascular development (*i.e*. increasing xylem cross sectional area) with increasing leaf area. All findings associated with effects of nutrient and water deficiency on leaf area reveal significance of the LA measurement.

There are some instruments for measuring LA, *i.e*. employing optical or laser scanner principles. However, measuring LA using these instruments may require leaf detachment off the plant; therefore, the growth analysis or physiological measurement can only be done once on any specific leaf. It is not possible to monitor LER over a period of time, for instance, in response to environmental stresses, such as during soil drying period, after initiation of flooding stress, or to compare LER during several cycles of light and dark periods in photoperiod studies.

On-site LA measurement using laser scanner has been practiced (Huang and Pretzsch, 2010). However, it must be noted that it is not easy to capture every single leaf images at perfect right angle, *i.e*. laser beam at position perpendicular to leaf surface. In spite of its arduous work, allometric measurement has been proven to be accurate and frequently used for validation of any other approaches, and vice versa.

LA can be estimated based on allometric and non-destructive measurements, *i.e*. based on L and/or W. Keramatlou *et al*. (2015) had developed models for LA estimation of walnut. LA estimation models had also been developed for chestnut (Serdar and Demirsoy, 2006), cucumber (Cho *et al*., 2007), faba bean (Peksen, 2007), chrysanthemum (Larsen and Nothnagl, 2008), ginger (Kandiannan *et al*., 2009), saffron (Kumar, 2009), eelgrass (Echavarría-Heras, 2010), rose (Gao *et al*., 2012), and *Jatropha curcas* (Pompelli *et al*., 2012).

Besides its most significant advantage of being non-destructive, the LA estimation models have several other advantages, including not depending on sophisticated Leaf Area Meter which may not be affordable by R&D institutions in developing countries, can be done at a location without access to electricity, easy to do (both in measuring L and W, and in using the models for estimating LA), and reliable. Most of the published models were using regression-based models to correlate between measured L, W, or LW to LA. Coefficient determination values (R^2) of recommended models were fallen within range of 0.96 to 0.99 (Serdar and Demirsoy, 2006; Peksen, 2007; Cho *et al*., 2007; Larsen and Nothnagl, 2008; Kandiannan *et al*., 2009; Kumar, 2009; Echavarría-Heras, 2010; Gao *et al*., 2012; Pompelli *et al*., 2012; Keramatlou *et al*., 2015). Therefore, in this study, LA estimation models with $R^2 > 0.96$ are considered as accurate and reliable.

Snap bean leaf is trifoliate leaf, consists of three leaflets: a symmetrical shape terminal leaflet and two side leaflets. Left-side leaflet are mirror image of right-side leaflet, and vice versa. A ste*P*=wise approach was employed in estimating LA of snap bean trifoliate leaves. Firstly, using Lt, Wt, or LtWt as predictor for LtA. Secondly, confirming that LtA of left and right-side leaflets are statistically similar. Thirdly, examining proportionality of LtA of terminal to those of side leaflets. Fourthly, as an objective of this research, exploring possibilities of minimizing directly measured predictors in LA estimation of snap bean trifoliate leaves.

Materials and methods

Snap bean used in this research was PV072 cultivar, a bushy-type snap bean preferred by farmers. More than two hundreds leaves were purposively collected from stock plants, to represent wide range of leaf sizes, from the smallest leaf with fully open leaf blade to the largest available leaf. Tondjo *et al*. (2015) sampled *Tectona grandis* leaves in similar approach to maximize variability in sizes along two categories of axes, *i.e* length and width of the leaves.

Each trifoliate leaf was partitioned into terminal, left-side, and right-side leaflets. Lt was measured based on the length of midrib and Wt was measured at the widest distance from side to side at direction perpendicular to the midrib. In this study, Lt varied from 2.1 cm to 15.5 cm, 1.5 cm to 14.1 cm, and 1.6 cm to 14.7 cm, for terminal, left-side, and right-side leaflets, respectively. Wt varied from 0.9 cm to 8.8 cm, 0.9 cm to 8.9 cm, and 0.9 cm to 9.0 cm for terminal, left-side, and right-side leaflets, respectively. LtA was calculated using area/weight ratio method. Each leaf was coded, scanned (using Epson L350 scanner/printer), and copied on standard 80-mg paper at real-size scale (100%). Images of all leaflets were traced, cut, and weighted for LtA calculation.

Pool of leaflet allometric data was alternately divided based on Lt into two subgroup: odd and even subgroup. Odd Subgroup was used for model development and Even Subgroup was used for validating the developed models. LtA estimation models were derived from linear, quadratic, and power regressions. It makes sense to force intercept to zero, since if Lt, Wt, or LtWt is zero, then LtA should also be zero. Five regression-based models were screened on their appropriateness for LtA estimation based on the R^2 value. Models with $R^2 < 0.96$ were dropped. Recommended models were decided based on the R^2 value and geometrical rationale.

Kandiannan *et al*. (2009) used data collected on subsequent years for development and validation of their models. Serdar and Demirsoy (2006) used data collected at the same year but from different location. They are all valid as long as data used were from different set of data for development and validation.

Every steps of effort on simplifying models for LA estimation through minimizing directly measured predictors were scrutinized, including confirming similarity of shape and size of left and right-side leaflets and examining proportionality of LtA of terminal to those of side leaflets. Size similarity between left and right-side leaflets is used as a base for making measurement only on either one, instead of both, of side leaflets. Consistent proportionality between terminal and side leaflets is used as an argument for using terminal leaflet to represent both side leaflets. At the end, the simplest but has the $R^2 > 0.96$ will be championed as recommended model for LA estimation of snap bean trifoliate leaf.

Results and discussion

Screening models for estimating LtA of the terminal leaflet: Use of linear regression for estimating LtA was not appropriate if only Lt or Wt used as predictor (Table 1). Simple linear regression tended to underestimate LtA at lower Lt or Wt value or for smaller leaves. LtA < 0 were observed if the regression lines were extrapolated to Lt < 3.519 cm or Wt < 1.859 cm. Negative value of LtA at Lt > 0 or Wt > 0 is not realistic; therefore, in this case, the linear regression models were not appropriate for LtA estimation model if Lt or Wt was used as predictor. Effort to rationalize LtA estimation by setting up a zero-intercept linear regression such that LtA is equal to zero (LtA = 0) if Lt = 0 or Wt = 0 created another drawback, *i.e*. the R^2 value dropped sharply from 0.9373 to 0.7938 if Lt was used as predictor and tumbled from $R^2 = 0.9340$ to 0.8043 if Wt was used (Table 1). Accuracy of linear regression for estimating LtA decreased if the intercept was set to zero.

Quadratic regression models performed better for estimating LtA based on Lt or Wt. The $R^2 = 0.9678$ for Lt and 0.9721 for Wt. Even without forcing intercept to zero, the intercept value had already closed to zero ($\alpha = 0.0758$) if Wt was used as predictor. Setting the intercept to zero, only slightly decreased the R^2 values, both for Lt and Wt, *i.e*. from 0.9678 to 0.9675 for Lt and unchanged at 0.9721 for Wt (Table 1). Performance of power regression was comparable to zero-intercept quadratic regression (Table 1).

Linear regression was a very accurate and reliable model for estimating LtA if the secondary level predictor of LtWt was used. The R^2 was 0.9955 and intercept was closed to zero ($\alpha = 0.031$). Setting the intercept to zero had no significant effect on the R^2 at 0.9955. Slope (β) of 0.5348 found from the zero intercept model using LtWt as predictor. Geometrically, it implied that the terminal leaflet occupied 53.48% of imaginary rectangular area of Lt x Wt.

Screening models for estimating LtA of the side leaflets: Appropriateness evaluation on selected regression models for estimating LtA of both (left and right) side leaflets disclosed

Table 1. Appropriateness of regression models for estimating surface area of terminal leaflet based on length, width, and length x width in snap bean

Predictor	Regression Model	Equation	R^2
Length (L)	Linear	LA = 4.8988L – 17.2400	0.9373*
	Zero-Intercept Linear	LA = 3.1099L	0.7938*
	Quadratic	$LA = 0.2555L^2 + 0.5884L – 1.7317$	0.9678
	Zero-Intercept Quadratic	$LA = 0.2782L^2 + 0.1698L$	0.9675
	Power	$LA = 0.2696L^{2.0394}$	0.9744
Width (W)	Linear	LA = 8.5935W – 15.9740	0.9340*
	Zero-Intercept Linear	LA = 5.6169W	0.8043*
	Quadratic	$LA = 0.8970W^2 + 0.3518W – 0.0758$	0.9721
	Zero-Intercept Quadratic	$LA = 0.9003W^2 + 0.3180W$	0.9721
	Power	$LA = 1.0875W^{1.9243}$	0.9850
Length x Width (LW)	Linear	LA = 0.5344LW + 0.0310	0.9955
	Zero-Intercept Linear	LA = 0.5348LW	0.9955
	Quadratic	$LA = 0.00005LW^2 + 0.5287LW + 0.1294$	0.9955
	Zero-Intercept Quadratic	$LA = 0.00002LW^2 + 0.5336LW$	0.9955
	Power	$LA = 0.5211LW^{1.007}$	0.9965

*) Dropped as candidates for LtA estimation model

similarity with that of terminal leaflet. For single direct measurement predictor of Lt or Wt, zero intercept quadratic and power regressions were appropriate models to be used for predicting LtA of both left and right-side leaflets (Tabel 2).

Based on the R^2 value, all evaluated regression models showed their appropriateness to be used in estimating LtA of both side leaflets if LtWt was used as predictor, the zero-intercept linear model had a more-sound geometrical foundation. The R^2 values of zero-intercept linear models were 0.9847 and 0.9871, for left and right-side leaflets, respectively. The slopes (β) were almost similar, 0.579 and 0.574 for left and right-side leaflets, respectively (Table 2). Comparing for the same model, the β value of terminal leaflet was smaller than those of left or right-side leaflets, indicating that terminal leaflet had a slightly slimmer shape than side leaflets.

In general, Pompelli *et al*. (2012) stated that if either W or L alone was used as the single leaf dimension, the power model predicted the LA with good accuracy at the expense heteroscedastic residual dispersion behavior. We observed similar residual behavior in our study, *i.e.* the variability of LtA was higher at larger Lt and Wt as predictors. The heteroscedastic behavior was much reduced if LtWt was used as predictor.

Similarity between zero-intercept quadratic and power regression models: The R^2 values of zero-intercept quadratic and power regression models in estimating LtA of snap bean leaflets based on single direct measurement parameters (Lt or Wt) were comparable. The power value of all power regression models were close to 2.0 as shown in Table 1 (for terminal leaflet) and Table 2 (for side leaflets). The power values for terminal leaflet were 2.0394 and 1.9243 for Lt and Wt, respectively; for left-side leaflet were 2.1003 and 1.7296, respectively; and for right-side leaflet were 2.0572 and 1.8951, respectively. These values indicated closeness of the power models to the zero-intercept quadratic model.

Moreover, it is interesting to visualize if the pattern of regression lines between the two models are similar. Comparison of the regression lines are presented in Fig. 1. It becomes clear that the regression lines are very similar. Therefore, if single parameter of Lt or Wt is used, both models are appropriate for estimating LtA.

Validation of selected models for LtA estimation: Appropriate models for estimating LtA were different between one-dimensional predictor (Lt or Wt) and two-dimensional predictor of LtWt. Selected model developed based on one-dimensional predictor is the zero-intercept quadratic regression model (Fig. 2); while for two-dimensional predictor of LtWt, selected model is the zero-intercept linear regression model (Fig. 3).

The selected models were validated using the Even Subgroup data. A perfectly valid model is established if slope (β) = 1.0 and R^2 = 1.0 which are almost impossible to achieve. In validating

Table 2. Appropriateness of regression models for estimating surface area of both side leaflets based on length, width, and length x width in snap bean

Predictor	Regression Model	Left Equation	R^2	Right Equation	R^2
Length (L)	Linear	LA = 5.071L - 16.235	0.9252*	LA = 5.086L - 16.378	0.9366*
	Zero-Intercept Linear	LA = 3.218L	0.7820*	LA = 3.233L	0.7917*
	Quadratic	$LA = 0.296L^2 + 0.582L - 1.892$	0.9597	$LA = 0.276L^2 + 0.792L - 2.319$	0.9682
	Zero-Intercept Quadratic	$LA = 0.326L^2 + 0.079L$	0.9592	$LA = 0.311L^2 + 0.185L$	0.9675
	Power	$LA = 0.267L^{2.1003}$	0.9771	$LA = 0.2914L^{2.0572}$	0.9812
Width (W)	Linear	LA = 8.369W - 13.712	0.9538*	LA = 8.445W - 14.014	0.9539*
	Zero-Intercept Linear	LA = 5.672W	0.8360*	LA = 5.701W	0.8339*
	Quadratic	$LA = 0.716W^2 + 2.117W - 2.545$	0.9834	$LA = 0.752W^2 + 1.792W - 1.958$	0.9856
	Zero-Intercept Quadratic	$LA = 0.835W^2 + 0.945W$	0.9823	$LA = 0.842W^2 + 0.894W$	0.9850
	Power	$LA = 1.525W^{1.7296}$	0.9332	$LA = 1.2141W^{1.8951}$	0.9910
Length x Width (LW)	Linear	LA = 0.569LW + 0.576	0.9852	LA = 0.561LW + 0.774	0.9879
	Zero-Intercept Linear	LA = 0.579LW	0.9847	LA = 0.574LW	0.9871
	Quadratic	$LA = -0.0008LW^2 + 0.645LW - 0.588$	0.9873	$LA = -0.0006LW^2 + 0.619LW - 0.138$	0.9892
	Zero-Intercept Quadratic	$LA = -0.0006LW^2 + 0.622LW$	0.9871	$LA = -0.0005LW^2 + 0.614LW$	0.9892
	Power	$LA = 0.642LW^{0.9724}$	0.9770	$LA = 0.5948LW^{0.9954}$	0.9953

*) Dropped as candidates for LtA estimation model

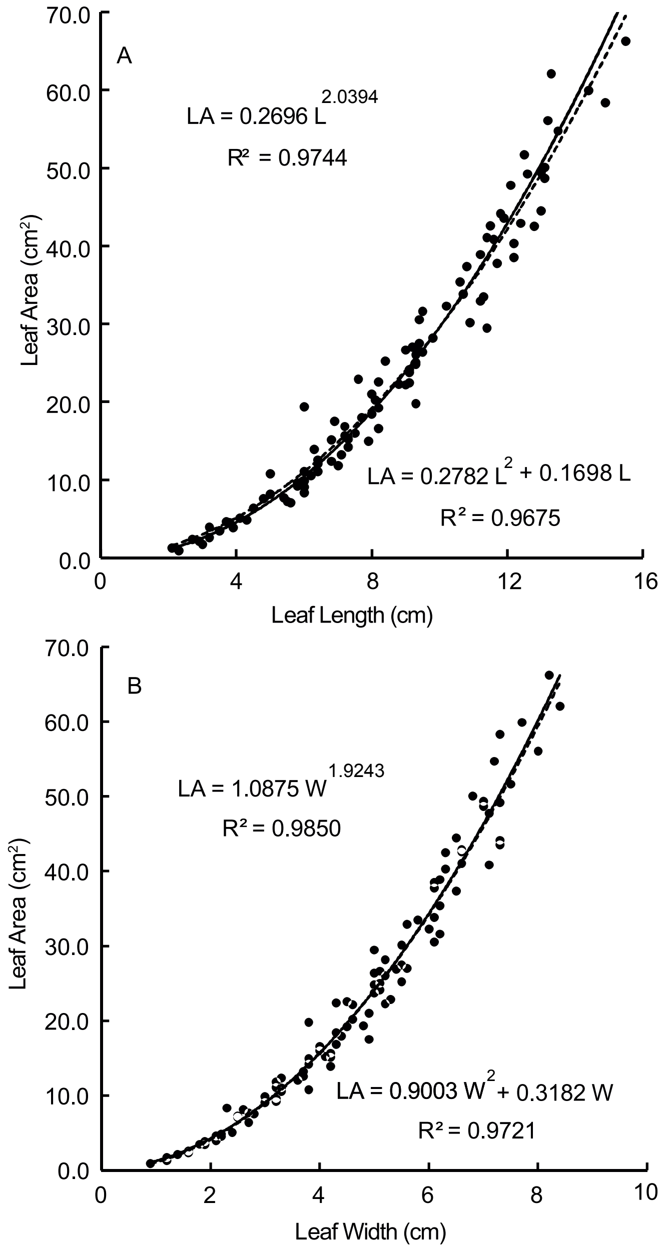

Fig. 1. Similarity between zero-intercept quadratic (broken line) and power regression (solid line) models for estimating surface area of terminal leaflets based on midrib length (A) and width of leaflet blade (B) in snap bean.

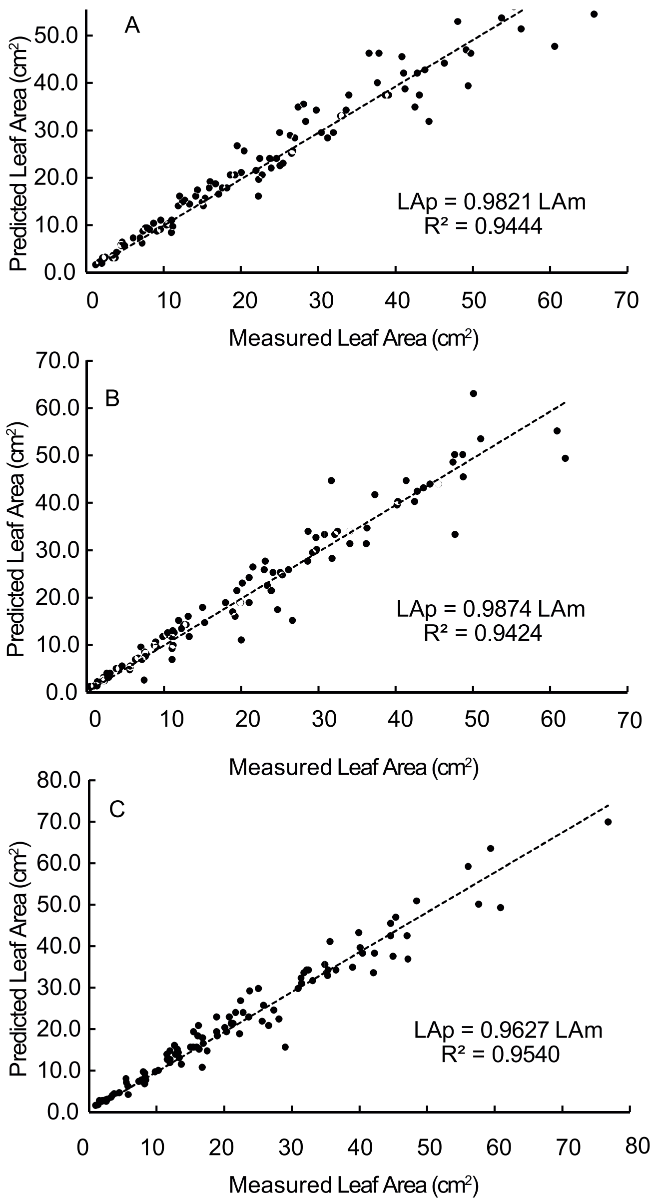

Fig. 2. Validation of zero-intercept quadratic model for estimating surface area of terminal (A), left-side (B), and right-side (C) leaflets based on midrib length in snap bean.

each model, a zero-intercept linear regression was used for correlating between LA calculated based on directly measured allometric data (LAm) of the Even Subgroup data (X axis) and predicted LA (LAp) based on the selected model, developed using the Odd Subgroup data (Y axis).

On validating zero intercept quadratic model using Lt for estimating LtA, it was found that the slopes were 0.9821, 0.9874, and 0.9627 for terminal, left-side, and right-side leaflets, respectively (Fig. 2). All of these slope values were less than 1.0, implying that the models tend to slightly underestimate LtA.

The slope values of zero intercept linear model using LtWt for estimating LtA were 0.9973, 0.9968, and 0.9744 for terminal, left-side, and right-side leaflets, respectively. Only slightly off the perfect correlation lines. Based on these values, compared to the models using single measurement of Lt, the model used LtWt as predictor performed better in estimating LtA with the R^2 values of 0.9925, 0.9820, and 9846 for terminal, left-side, and right-side leaflets, respectively. All of the R^2 values were higher than 0.96.

The selected models using one-dimensional measurement were less accurate. The R^2 values were 0.9444, 0.9424, and 0.9540 for terminal, left-side, and right-side leaflets, respectively (Fig. 2). However, these models have obvious advantage of rapid measurement and more practical for large sample experiments.

Symmetry between left and right-side leaflets: Visually, shape of left-side leaflet is a mirror image of the right-side leaflet. Therefore, it is worth to assess if they are of equal size. If the size is statistically similar, therefore total area of snap bean leaf can be estimated by only measuring one of the two side leaflets and then multiplied by two plus the size of terminal leaflet. This will save one-third of the work and time. In this study, it was found that the two side were statistically similar in size (Fig. 4). Degree of statistical similarity should increase if larger number of leaflets were sampled.

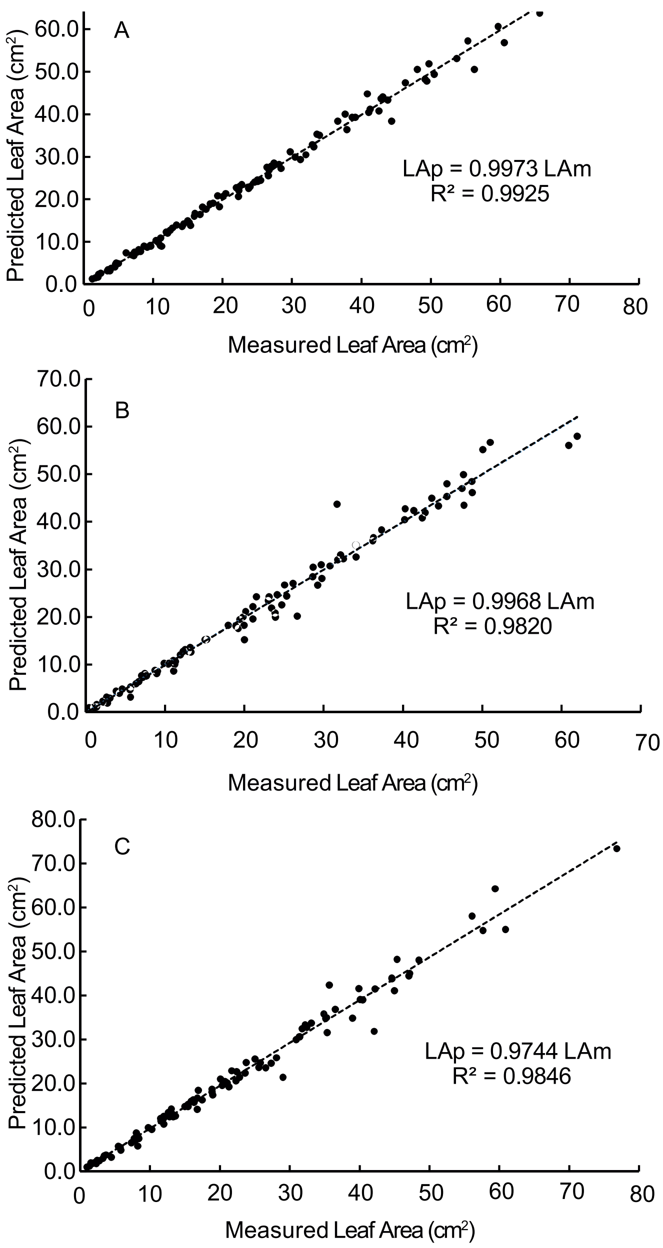

Fig. 3. Validation of zero-intercept linear model for estimating surface area of terminal (A), left-side (B), and right-side (C) leaflets based on the length x width in snap bean.

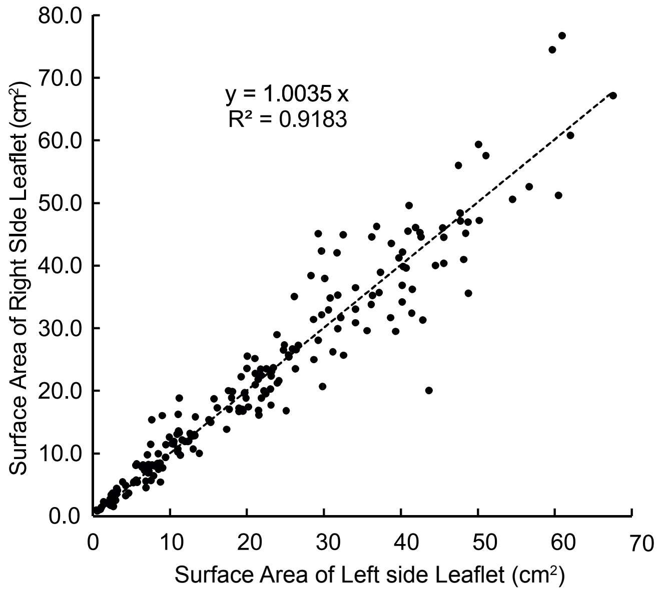

Fig. 4. Comparison of surface area between left and right leaflets in snap bean.

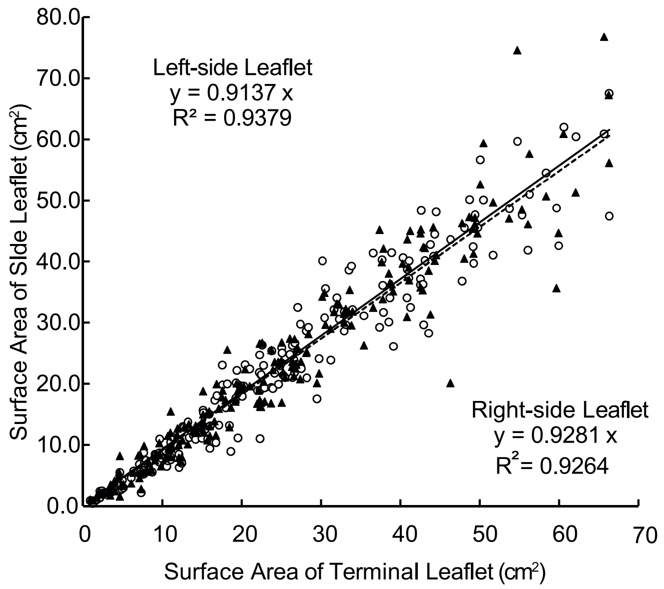

Fig. 5. Proportionality between surface area of terminal to left-side (broken line) and right-side (solid line) leaflets in snap bean.

Actually, there was no couple of side leaflets that has exactly the same size. Some have slightly larger left-side leaflets and some other have larger right-side leaflets, especially at larger leaves as shown by higher heteroscedastic residual dispersion behavior. Larger number of samples reciprocally compensate the slight difference in size of both sides. Since y/x = 1.0035, then averaged LA of right side leaflet was only 0.35 percent larger than that of left side leaflet.

Terminal/side leaflet ratio: In most cases, there are relatively proportional size between terminal and side leaflets at all stages of snap bean leaf development. In other words, growth rate of terminal leaflet is comparable to that of side leaflets. If size proportion between terminal and side leaflets can be consistently proven, then single measurement or dimension of terminal leaflet can reliably represent the total LA of the designated snap bean leaf. Fig. 5 exhibits the proportional relationship between terminal and both side leaflets. The R^2 values were 0.9379 and 0.9264 for left and right-side leaflets, respectively. Side/terminal leaflet (y/x) ratio were 0.9137 and 0.9281 for left and right-side leaflet, respectively.

Simplifying models by minimizing measured predictor(s) for estimating trifoliate LA: The best possible option in any measurement activities is associated with accuracy, simplicity/practicality, speed, cost, and level of disturbance to the subject. However, in reality, compromise is unavoidable. Researchers frequently have to negotiate the accuracy for faster measurement since the subject studied changes rapidly, go for simple and inexpensive measurement technique due to limited budget or lack of sophisticated instrument, or stick on simple but non-destructive approach in studying living organism or bioprocess even though a sophisticated instrument is available but it cause disruption to the organism or to the process.

Knowing that: (a) Lt correlates with LtA, (b) LtA of terminal leaflet is proportional to LtA of side leaflets, (c) LtA of left and right-side leaflets are statistically similar; then, there should be a correlation between midrib Lt of terminal leaflet and LA of the designated trifoliate leaf. Using zero-intercept quadratic regression model, the R^2 value was 0.9287 in estimating LA using single measurement of Lt as predictor (Fig. 6). This approach is considered to be a fast measurement for estimating LA, but it may not satisfy for those who expected higher accuracy.

For increasing accuracy, total Lt of terminal and side leaflets (ΣLt) was used as predictor for trifoliate LA. This ΣLt approach represents all three leaflets of snap bean trifoliate leaf, but it takes three measurements for each leaflet. Therefore, obviously, it will be three times slower to execute. However, this effort has paid off, in term of increase in accuracy. The R^2 value increased to 0.9681 (Fig. 7), compared to 0.9287 based on solely measuring Lt of terminal leaflet.

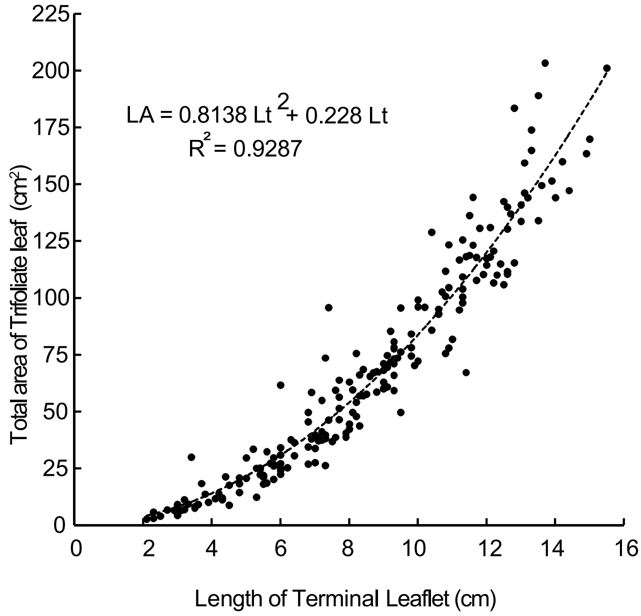

Fig. 6. Use of midrib length of terminal leaflet as a single measurement for estimating total leaf area of trifoliate leaf in snap bean.

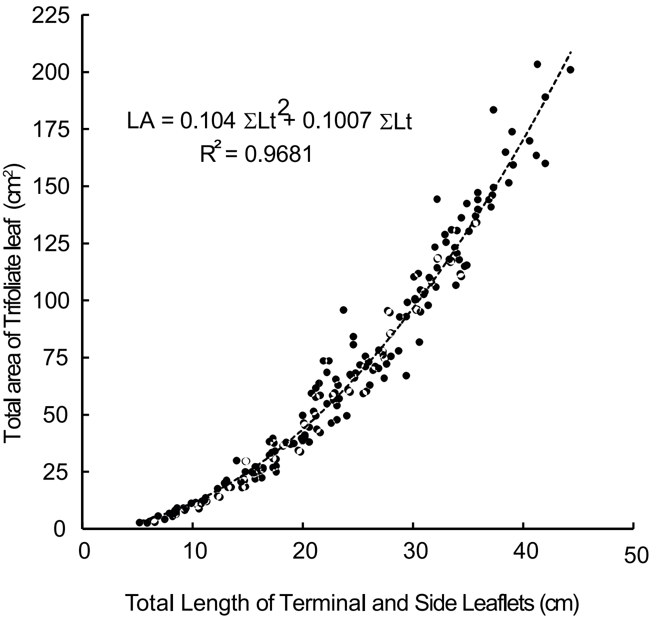

Fig. 7. Use of total midrib length of all three leaflets for estimating total leaf area of trifoliate leaf in snap bean.

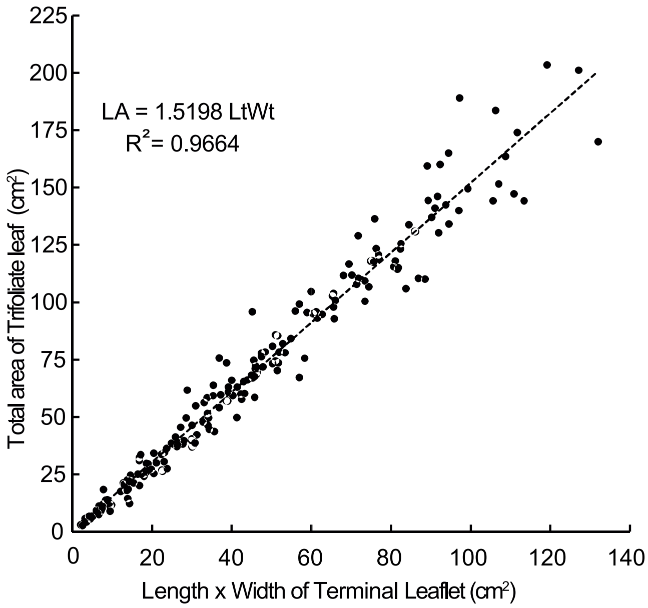

Fig. 8. Use of the LW of terminal leaflet for estimating total surface area of trifoliate leaf in snap bean.

It is equally accurate if LW of terminal leaflet was used for estimating LA of snap bean trifoliate leaf. The R^2 value of 0.9664 (Fig. 8) was comparable to the R^2 value of 0.9681 when ΣLt was used for estimating the trifoliate leaf (Fig. 7). However, LW only requires two measurements; thus, it can be executed in shorter time than the three measurements of the ΣLt approach.

A simple procedure for non-destructive, inexpensive, and accurate LA estimation in snap bean can be developed and validated by screening many regression-based models for finding the most appropriate model. There is take-and-give decision between more practical and time considerations with expected accuracy level. After exercising: (a) screening linear, quadratic, and power regression models, (b) considering option of forcing intercept to zero for making a more geometrically-sound model, (c) testing symmetry between left and right-side leaflets, and (d) determining proportionality between terminal and side leaflets; we recommend the practical, non-destructive, inexpensive, and accurate area estimation for trifoliate leaf of snap bean is LA = 1.5198 LtWt of terminal leaflet.

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