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Accuracy of using leaf blade length and leaf blade width measurements to calculate the leaf area of *Solanum aethiopicum* Shum group

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Abstract

Leaf area is an important parameter when determining growth response under normal as well as stressed environments. No attempt had been made to come up with an affordable but accurate alternative of measuring leaf size in research neglected leafy vegetable crops. Other techniques such as use of leaf area meters are either destructive, expensive or both. A study was conducted to determine leaf area in like-shaped leaves of research neglected crop plants, taking case of *S. aethiopicum* Shum group (SAS) germplasm. Data was collected on 552 individual plants (including pure lines and hybrids) at eight weeks after planting where a third fully open leaf from top of each plant was considered. Leaf blade length (LBL) and leaf blade width (LBW) were linearly measured while leaf area (LA) was measured using a leaf area meter. This was followed by correlation and regression analysis of LA with LBL, LBW, and LBL + LBW. Correlation coefficients at $p < 0.001$ ranged between 0.84 and 0.92, 0.79 and 0.88, 0.86 and 0.95 for total germplasm, pure lines and hybrids, respectively. The coefficient of

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determination (R^2) ranged between 0.72 and 0.92. The best prediction for LA was obtained with hybrid plants (LA = -165.82 + 5.38LBL + 16.17LBW) at R^2 of 92%. The implication is that we can accurately and affordably predict LA from duo measurements of LBL and LBW in SAS as well as in other crops having similar leaf shapes.

Keywords: Biophysics, Ecology, Plant biology

1. Introduction

Leaves are the largest proportion of the total canopy surface in most plants [1]. They are the main site for physiological processes like photosynthesis and transpiration [1, 2]. They are responsible for regulating gas exchange [3] and hosting various metabolic processes which influence crop growth and yield [1, 2, 3]. The canopy contributed by leaves also regulate weed abundance through a crop−weed competition tendency, crop water use efficiency and drought tolerance, and soil erosion control by regulating rainfall drop impact [4].

In leafy vegetables like SAS; leaves are the major edible parts hence making leaf area paramount [5, 6, 7, 8, 9, 10, 11, 12]. Leaf area is a very important component for determining plant growth rate and yield as it can be captured at different stages [10, 13, 14]. Leaf size is therefore a parameter that should be accurately measured in leafy vegetables as it influences leaf yield in these crops [9, 15]. There are different methods of determining leaf area and these can either be destructive or non-destructive [2]. The destructive methods are undesirable and involve striping the plant canopy and measuring the foliage of the plant canopy sample, using laser scanners, cameras and conventional optical planimeter [1, 16, 17]. Additionally, the measuring can accurately be done by sketching out a leaf on a squared paper (grid counting method) [16]. Despite the accuracy of the grid counting method, it is very tiresome, time consuming and may not be applicable to large leaf sizes and where a high number of samples is involved.

Alternative to the tiresome grid counting method and the destructive techniques is the imaging method where pictures of the leaf sample are taken and automatically calculated by the machine [18]. This method requires sophisticated equipment which is very expensive and not available in most developing countries [2, 18]. In addition, the sophisticated imaging methods are not suitable for field usage since some need continuous electric power supply, and others which can operate on lithium battery cells can still not be sustained for long because of need for frequent battery replacement [13]. Nonetheless, portable laser leaf area meters are rechargeable [2] and this gives opportunity for their use in field-based optimization of suitable models that relate accurate leaf area to linear measurements of leaf blade length and leaf blade width.

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In *Mespilus germanica*, a leaf area linear model having leaf width (LW) as the independent variable (A = 1.81 + 0.68 LW) provided an accurate estimate of leaf area with 98.1 percent of leaf area variation being explained by the LW [19]. Yeshitila and Taye [20] used a standard method for determining leaf area for different vegetable crops through multiple-regression analysis in order to establish a close relationship between actual and predicted leaf area [20]. Elsewhere, research on leaf area measurement in *Rosa hybrida* L. recommended a linear model where leaf blade length and leaf blade width provided the most accurate leaf size estimate [21]. This agreed with the results obtained by [22] in Cabernet-Sauvignon grapevine leaves. In another study, two greenhouse plants (tomatoes and Gerbera) were evaluated using different models and the correlation between predicted and actually measured leaf area was significantly high when both leaf blade length and leaf blade width were used [23]. When a study was carried out in *S. macrocarpon*, a model: Leaf area = 1.06 + 0.4731L was recommended for predicting leaf area [24].

Different models were developed for the different crops that vary in leaf shape [18, 19, 20, 24, 25, 26]. Inadequate attempt had been made to come up with an affordable but accurate alternative of measuring leaf size in research neglected leafy vegetable crops [21, 27, 28]. Leaf shape in *S. aethiopicum* morphological groups (Gilo, Shum, Kumba and Aculeatum) and their wild progenitor (*S. anguivi*) is generally similar [7, 29]. Thus, any model for accurate measurement of leaf area using one of the morphological groups can be robustly applied across the subspecies. This study used the leafy type, SAS, owing to its appreciable diversity among varieties within the group [6, 29, 30], so as to leverage robust applicability of resulting model(s) across subspecies [5, 30], and species with similar leaf shapes. It is thus hypothesized that LA can be accurately and affordably estimated based on LBL and/or LBW. This study was aimed at establishing a reliable model that can be used to determine LA in like-shaped leaves of research neglected crop plants, taking case of SAS germplasm. Specifically, we determined the correlation among LBL, LBW and LA, and the predictive accuracy of leaf area LBL and LBW.

2. Materials and methods

Eighteen pure lines and 26 F1 hybrids of *S. aethiopicum* Shum group were planted in a screen house in a completely randomized design at Department of Agricultural and Biological Sciences, Uganda Christian University, Mukono, Uganda. Twelve plants per variety were used. Direct sowing into plastic pots (10-kg capacity for potting substrate) each filled with 10kg of a loamy soil and poultry manure (3:1) was carried out followed by thinning to one plant per pot. The plants were optimally watered throughout the experiment.

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Data collection was carried out on individual plants at 8 weeks after planting (WAP). A 3rd fully open leaf from top each plant was considered. Variables namely leaf blade length in centimeters (cm), leaf blade width (cm) were measured using a ruler while leaf area (LA) was measured using a leaf area meter, model AM350 (ADC Bioscientific Ltd, Globol house, Geddings Road, Hoddesdon, Herts, EN11 NT, UK) as earlier applied in our previous study [9]. Scatter plots between LBL and LBW, and LBL and LA, and LBW and LA were made. The correlation analysis was followed by regression analysis of LA with LBL, LBW, LBL plus LBW and LBL multiplied by LBW using Genstat. The leaf area, leaf length and leaf width of 552 leaves (12 leaves per accession) of 18 pure line and 26 hybrids of SAS accessions were used to establish the models.

3. Results

3.1. Correlation analysis

Comparisons between meter-measured leaf area (LA) and leaf blade width (LBW), meter-measured LA and leaf blade length (LBL), and LBL with LBW were made. The results showed a high degree of correlation ($p < 0.001$) with Pearson's correlation coefficients (r) of 0.9221 (LA vs LBW), 0.8756 (LA vs LBL), and 0.8423 (LBL vs LBW) for all evaluated material lumped up, respectively. For pure lines, $r = 0.8790$ (LA vs LBW), $r = 0.8483$ (LA vs LBL) and $r = 0.7939$ (LBL vs LBW). The correlation coefficients for hybrids were higher than for pure lines, that is, $r = 0.9468$ (LA vs LBW), $r = 0.8878$ (LA vs LBL) and $r = 0.8611$ (LBL vs LBW) respectively. The grand mean measurements (for all evaluated germplasm) was 224.9cm^2 (LA using leaf area meter), 21.15cm (LBL) and 16.95cm (LBW). For pure lines, the mean measurements were 208.7cm^2 (LA), 20.3cm (LBL) and 16.1cm (LBW); while 236.7cm^2 (LA), 21.8cm (LBL) and 17.5cm (LBW) was obtained for hybrids.

3.2. Fitted and observed relationships with 95% confidence interval

A linear relationship between leaf area and leaf blade width and length for both fitted and observed values was obtained when all germplasm was lumped up (Fig. 1). Similar trends were observed for pure lines (Fig. 2) and hybrids (Fig. 3) as individual groups. The line of best fit for hybrid plants (Fig. 3) shows a higher coherence than that of pure lines (Fig. 2) plus that of both pure lines and hybrids (Fig. 1). There was a higher degree to which scatter points for case of hybrids (Fig. 3) were clustered along the line of best fit and with a higher correlation. Furthermore, the relationship between leaf area and leaf blade width in hybrid lines proved to be more suited than the relationship between leaf area and leaf blade width in pure lines.

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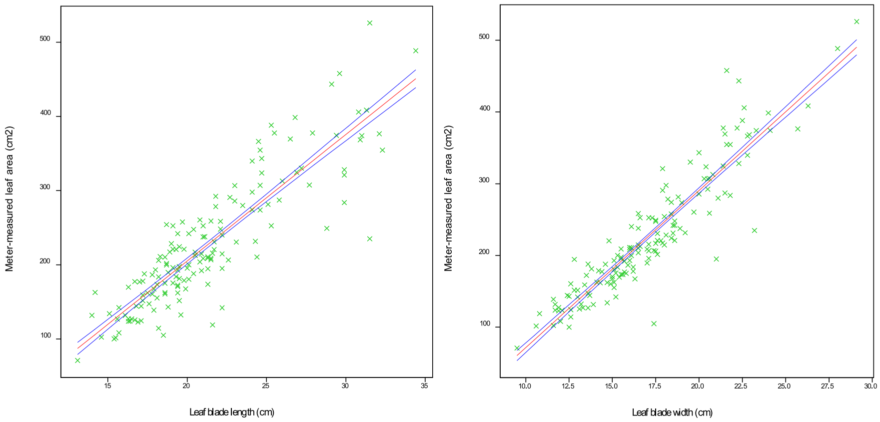

Fig. 1. The relationship between the meter measure leaf area and leaf blade length (left) and leaf blade width (right) for both pure lines and hybrids.

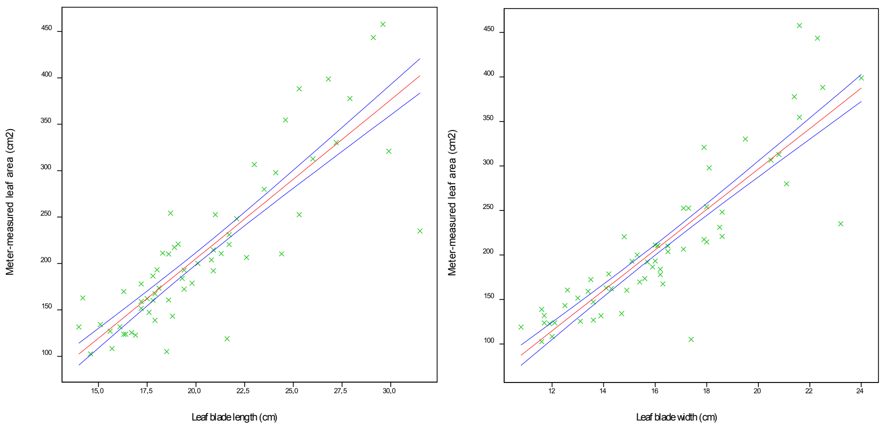

Fig. 2. The relationship between the meter measure leaf area and leaf blade length (left) and leaf blade width (right) for pure lines.

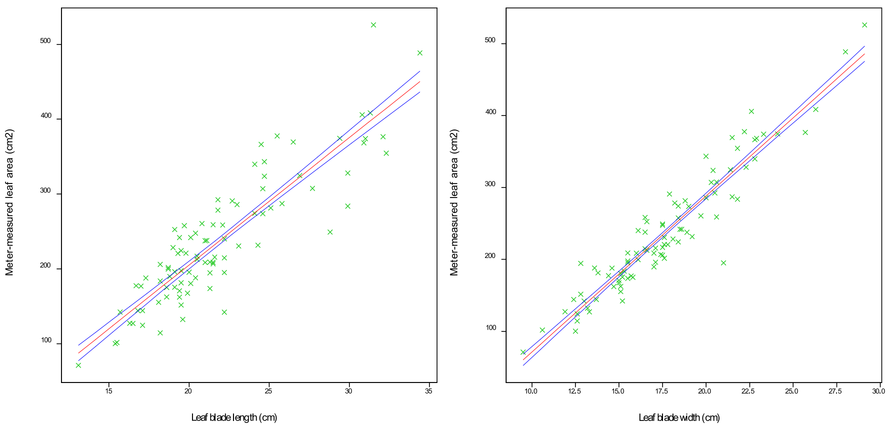

Fig. 3. The relationship between the meter measure leaf area and leaf blade length (left) and leaf blade width (right) for hybrids.

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3.3. Regression analysis

Different possible models fit for LA were tested and the results were as summarized in the tables (Tables 1, 2, and 3). Table 1 presents results of the linear regression analysis carried out on both pure lines and hybrids. There are significant ($p < 0.001$) linear relationships between meter-measured LA with LBL (Mode 1), LBW (Model 2) and LBL + LBW (Model 3). The LA models involving LBL, LBW and LBL + LBW explained 77%, 85% and 88% of the variation in *S. aethiopicum.* Model 3 with a coefficient of determination (R^2) of 0.88 emerged as a superior model.

Table 2 presents results of the linear regression analysis carried out on pure lines. The results also showed significant ($p < 0.001$) linear relationships between

Table 1. Estimates of parameters for the three alternative predictive models for leaf area in *S. aethiopicum* Shum for both pure lines and F1 hybrids.

Parameter	Estimate	s.e	t-value	significance	R^2
Model 1: LA = a_1 + b_1LBL + e					
Intercept, a_1	-136.21	9.61	-14.17	***	0.77
Slope, b_1	17.065	0.442	38.63	***	
Model 2: LA = a_2 + b_2LBW + e					
Intercept, a_2	-147.66	7.55	-19.55	***	0.85
Slope, b_2	21.919	0.432	50.76	***	
Model 3: LA = a_{34} + b_3LBL + b_4LBW + e					
Intercept, a_{34}	-172.41	7	-24.64	***	0.88
Slope, b_3	6.639	0.579	11.47	***	
Slope, b_4	15.098	0.706	21.39	***	

*** significance at 0.05, 0.01 and 0.001 error margin, respectively. LA, leaf area; LBL, leaf blade length; LBW, leaf blade width; a, intercept; b, slope; e, random error; R^2, coefficient of determination.

Table 2. Estimates of parameters for the three alternative predictive models for leaf area in *S. aethiopicum* Shum pure lines.

Parameter	Estimate	S.e	t-value	significance	R^2
Model 1: LA = a_1 + b_1LBL + e					
Intercept, a_1	-137.44	16.525	-8.32	***	0.72
Slope, b_1	17.1293	0.7948	21.55	***	
Model 2: LA = a_2+ b_2LBW + e					
Intercept, a_2	-158.01	15.1956	-10.4	***	0.77
Slope, b_2	22.7292	0.9166	24.8	***	
Model 3: LA = a_{34} + b_3LBL + b_4LBW + e					
Intercept, a_{34}	-189.58	13.591	-13.95	***	0.83
Slope, b_3	8.218	1.009	8.14	***	
Slope, b_4	14.374	1.292	11.12	***	

*** significance at 0.05, 0.01 and 0.001 error margin, respectively. LA, leaf area; LBL, leaf blade length; LBW, leaf blade width; a, intercept; b, slope; e, random error; R^2, coefficient of determination.

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Table 3. Estimates of parameters for the three alternative predictive models for leaf area in *S. aethiopicum* Shum Hybrid lines.

Parameter	Estimate	S.e	t-value	Significance	R^2
Model 1: LA = a_1 + b_1LBL + e					
Intercept, a_1	-135.5	12	-11.29	***	0.79
Slope, b_1	17.031	0.536	31.75	***	
Model 2: LA = a_2 + b_2LBW + e					
Intercept, a_2	-145.68	8.1	-17.99	***	0.90
Slope, b_2	21.7	0.448	48.44	***	
Model 3: LA = a_{34} + b_3LBL + b_4LBW + e					
Intercept, a_{34}	-165.82	7.68	-21.58	***	0.92
Slope, b_3	5.379	0.662	8.12	***	
Slope, b_4	16.166	0.791	20.43	***	

*** significance at 0.05, 0.01 and 0.001 error margin, respectively. LA, leaf area; LBL, leaf blade length; LBW, leaf blade width; a, intercept; b, slope; e, random error; R^2, coefficient of determination.

meter-measured LA with LBL (Mode 1), LBW (Model 2) and LBL + LBW (Model 3). The LA models involving LBL, LBW and LBL + LBW explained 72%, 77% and 83% of the variation in *S. aethiopicum.* Model 3 with a coefficient of determination (R^2) of 0.83 still emerged as a superior model.

Table 3 presents results of the linear regression analysis carried out on hybrids. The results showed significant ($p < 0.001$) linear relationships between meter measured LA with LBL (Mode 1), LBW (Model 2) and LBL + LBW (Model 3). The LA models involving LBL, LBW and LBL + LBW explained 79%, 90% and 92% of the variation in *S. aethiopicum*. Model 3 with a coefficient of determination (R^2) of 0.92 emerged as a superior model. Despite the significance portrayed in each case, it was noted that all the three models performed best for hybrids followed by pure lines, and then a combination of both with a high level of significance at $p < 0.001$. Model 3 had the largest coefficient of determination followed by model 2 while Model 1 had the lowest coefficient of determination in pure lines, hybrid lines and a combination of both hybrids and pure lines.

4. Discussion

The relatively weak correlation between leaf length and leaf blade width does not give enough evidence that an increase in leaf length would actually cause an increase in leaf blade width; though earlier studies had shown strong correlations [8, 9]. Strong positive correlation between meter measured and calculated leaf area implies that leaf area (LA) can be predicated using a linear measurement hence the possibility to use either LBL, LBW or LBL + LBW [21, 23, 25, 26, 31], to obtain relative accurate values of leaf area in *S. aethiopicum*. However, LBW was more correlated than LBL implying that leaf blade width is a better option for estimating actual leaf

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area as it gave more accurate results [19, 26]. It was still realized that the correlations with hybrids were higher than the correlations of pure line and both (pure lines and hybrids).

The relationship in the graph indicates that leaf area is dependent on leaf blade length and width. The more suited line of best fit for leaf blade length implies that leaf blade length predicts leaf area better than leaf blade width. Furthermore, the relationship between leaf area and leaf blade width in pure lines seemed to be more suited than the relationship between meter-measured leaf area and leaf blade length. This means that the leaf blade width provided a more accurate estimate of actual leaf area. However, a combination of both length and width gave the best leaf area estimates. This is in conformity with findings obtained in *Hymenaea courbaril* L. by [16].

Despite the fact that all the 3 models performed well, model 3 performed better than model 1 and 2. Model 3 (for instance for pure lines; LA = -189.58 + 8.218LBL +14.374LBW) had the largest coefficient of determination hence predicted the leaf area more perfectly than the rest. This means for a better prediction one needs both LBL and LBW; as previously demonstrated by [20, 25]. It implies that in pure lines of *S. aethiopicum* Shum, if LBL is held constant, every 1 cm of LBW contributes to actual leaf area by 14.374 cm^2. Rouphael et al. [32], while working on *Rosa hybrida* L., similarly emphasized the use of both leaf blade length and width (in their study, a product of the two parameters was used; instead of "additions") as being more accurate in leaf area estimation. Similar findings were also reported by [33] in eggplant. Aside the pure lines, use of both LBL and LBW also resulted in better leaf area estimation accuracy than use of LBL or LBW singly. However, the accuracy of determination for actual leaf area was higher for all the three model options (LBL, LBW or $LBL + LBW$) in F1 hybrids than pure lines; as earlier observed by [2]. Hybrids (particularly F1s) tend to be uniform in all aspects including leaf shape; enabling consistent measurements (reduced variation) which results in better accuracy.

5. Conclusion

Stronger correlation exists between meter-measured leaf area and leaf blade width than with leaf blade length. For pure lines, LBW predicts LA at R^2 of 77% while for hybrids, the R^2 is 90%. Putting pure line and plants together, LBW estimates LA at R^2 of 85%. The actual LA in *S. aethiopicum* Shum group can however, be best predicted from a combination of LBL and LBW measurements using the models: LA = -189.58 + 8.22LBL + 14.37LBW for pure lines at R^2 = 83%; LA = -165.82 + 5.38LBL +16.17LBW for hybrids at R^2 = 92%; and LA = -172.41 + 6.64LBL +15.10LBW for both pure lines and hybrids at R^2 = 88%. Whereas good prediction is attainable for all types of *S. aethiopicum* that are similarly shaped,

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the best accuracy is achievable for uniform genotypes such as F1 hybrids. These estimates were made under uniform conditions of screen house; thereby requiring follow-up studies in the field.

Declarations

Author contribution statement

Godfrey Sseremba: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mildred Julian Nakanwagi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Nahamya Pamela Kabod: Performed the experiments.

Michael Masanza: Contributed reagents, materials, analysis tools or data.

Elizabeth Balyejusa Kizito: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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