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Leaf area estimate of *Pennisetum glaucum* by linear dimensions

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ABSTRACT. Leaf area measurements are of the main parameters used in agronomic studies to evaluate plant growth. The current study used a non-destructive method based on linear leaf dimensions (length and width) to select the regression model to estimate millet (*Pennisetum glaucum*) leaf area. For two millet genotype (IPA BULK 1 BF and ADR 300) 128 randomly-chosen leaves were measured at different vegetative growth stages. Measures of length and width of each leaf were made using digital calipers. Leaf area was measured using a gravimetric method. The best-fit leaf area estimation model was selected via linear, potential and gamma regression models. Leaf area values varied from 3.02 to 209.21 cm². The average value was 95.31 cm². The potential regression model exhibited lower residual sum of squares and Akaike's information criterion and similar determination coefficient and Willmott index. Thus, potential regression was more efficient in explaining the leaf area of millet, independent of the genotype, when compared to other models evaluated in this research. Length (L) and width (W) could be used in the following potential regression model $\hat{Y} = 0.879LW^{0.971}$ to estimate millet leaf blade.

Keywords: leaf blade; millet; modeling; non-destructive method.

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Introduction

Millet (*Pennisetum glaucum* (L.) R. Br.) is a Poaceae species of African origin, that is cultivated in several Brazilian states. It is a short life-cycle plant with multiple purposes, and can be used as soil cover in no-tillage cultivation, as forage in direct grazing systems, silage and cultivated as grain for human and animal consumption (Pedroso, Monks, Ferreira, Tavares, & Lima, 2009). Millet has high nutritive value and, in comparison to other important crops such as sorghum and maize, has a notably high crude protein content (Ullah, Ahmad, Khaliq, & Akhtar, 2017) and potential to produce medium-level volumes of biomass, especially in dryland areas due to its relatively low water demand (Nagaz, Masmoudi, & Mechila, 2009). Thus, millet is a promising alternative crop in semiarid Brazilian regions.

Determination of leaf area is an important tool for studying transpiration intensity, net assimilation rate, leaf area ratio, specific leaf area and leaf area index (Schmildt, Amaral, Schmildt, & Santos, 2014), as well as to quantify leaf damage caused by diseases and pests. Additionally, leaf area estimates are efficient indicators of solar radiation interception by foliage, which in turn affects the quantity and quality of produced biomass (Flumignan, Adami, & Faria, 2008). Currently, several destructive and non-destructive methods have been used to estimate millet leaf area, all with varying levels of precision (Silva, Costa, Caputti, Galzerano, & Ruggieri, 2013). Indirect and non-destructive methods allow successive fast and precise analysis measurements from the same plant (Toebe, Cargnelutti Filho, Loose, Heldwein, & Zanon, 2012).

Development of regression models using linear leaf measurements to predict individual leaf area has been shown to be very useful for morphogenic studies (Achten et al., 2010). Application of such mathematical models has advantages over the use of destructive leaf area methods, mainly because they are simple to use under field conditions and do not require the destruction of the study plant. Several methodologies using real leaf area and leaf dimensions have been created for forage and crop plants: *Urochloa mosambicensis* (Leite, Lucena, Sá Júnior, & Cruz, 2017), *Brachiaria brizantha* genotype Xaráes and *Panicum maximum* genotype Massai (Silva et al., 2013), corn (Vieira Junior et al., 2006), coffee (Antunes, Pompelli, Carretero, & DaMatta, 2008), sunflower (Aquino, Santos Júnior, Guerra, & Costa, 2011), passion fruit (Morgado, Bruckner, Rosado, Assunção, & dos Santos, 2015). In all of these the authors reported very precise leaf area estimates.

Although millet is a well-studied plant, there has, so far, been no information reported on its leaf area. Consequently, the aim of this study was to define the best model to estimate the-millet leaf area, using non-destructive methods based on leaf blade maximum length and width.

Material and methods

Research was carried out under greenhouse conditions across 90 days at the Federal Rural University of Pernambuco (Serra Talhada County Campus), in the semiarid region of Pernambuco State, northeastern Brazil. The greenhouse was located at 07° 57' 01" S, 38° 17' 53" E, at an elevation of 523 meters. According to Köppen and Geiger (1928), the climate condition is a BSw_h' with rainy season during the summer, starting in November and ending in April.

Plastic 9.95 dm³ pots were filled with Argisil (soil collected from a cultivated pastureland on the Federal Rural University of Pernambuco Serra Talhada campus. In recent years this area has been under *Urochloa mosambicensis* pasture. Soil was collected from the first 20 cm, and then crushed, homogenized, sifted (2.0 mm) and used to fill the pots. At the same time a soil sample was collected and sent to the university soil laboratory, where analysis revealed the following attributes: pH (water) = 7.20; P (extractor Mehlich I) = 40 mg dm⁻³; K⁺ = 0.45; Ca²⁺ = 5.3; Mg²⁺ = 1.1; Na⁺ = 0.06; Al³⁺ = 0.0; H⁺ = 1.23; SB = 6.91; CTC = 8.14 (cmolc dm⁻³) and V = 84.89%.

Three seeds of two genotype of millet (*Pennisetum glaucum*) were planted in each pot (three seeds per pot). After germination, each pot was thinned to one seedling. During the first 30 days after planting the pot were maintained in field capacity to avoid the seedlings suffering hydric stress.

A gravitational method was used to control the pot water levels, which were daily monitored for weight of pot + soil + water. Pots were recharged the water loss daily by evapotranspiration in each pot, using methodology described by Casaroli and Van lier (2008).

Experimental design was randomized, with two millet genotypes (IPA BULK 1BF and ADR 300), and three replications per treatment. During the experiment no fertilization occurred and the invasive plants (weeds) were removed weekly. The day on which all seedlings had emerged was counted as the first Day After Emergence (DAE). Plants were then evaluated every 15 days (15, 30, 45, 60, 75 and 90 DAE), totaling six evaluations. For each evaluation plants were harvested and carried to forage studies laboratory, where the samples were fractioned into the following morphological components: dead material, stem, and leaf blades. Leaf area was determined after the division of morphological components.

Randomized collection of 128 green leaf blades, free of damage, diseases, and pests were conducted. Leaves were collected in all growth stages, following the recommendations to include different growth phases made by Schmidt et al. (2014) and Leite et al. (2017). Leaves were numbered from 1 to 128 and then, using digital calipers, the length (L) and width (W) was measured (cm) for each leaf blade. Length was measured along the central vein of the leaf, considering as leaf area from the insertion point of the blade with the ligule to the leaf apex. Width was measured at median part of the leaf blade, perpendicular to the leaf central vein. The length and the width were multiplied determining the product in cm². Each leaf was then spread over millimetered graph paper, and the outline traced following a method given by Leite et al. (2017). Using scissors, the area of the millimeter graph paper covered by the outline was then cut out and weighed on an electronic scale.

From the same paper a 10 cm x 10 cm square was cut, equivalent to 100 cm², weighing 0.630 g. It was therefore possible to calculate the proportional leaf area of each leaf for each millet genotype. The best-fit model for predicting leaf area was selected via a mathematical model. Three models were applied: linear with normal distribution, assuming that dependent variable response lies in the range $(-\infty, \infty)$, linear with gamma distribution, assuming that dependent variable response lies in the range $(0; \infty)$, and a power model (Table 1).

Table 1. Regression models to explain the leaf area of millet in relation the explanatory variable, product of length by width (L x W).

Models	Explanatory Variables
	L x W
Linear	$Y_i = \beta_1 LW_i + \varepsilon_i$
Gamma	$Y_i = \beta_1 LW_i + \varepsilon_i$
Power	$Y_i = LW_i^{\beta_1} \varepsilon_i$

Were, Y_i is the i-th leaf area; LW, the product between length and width of i-th leaf blade and ε_i the i-th error related to leaf area, which ε_i exhibiting the mean normal distribution and variance constant $\sigma^2 > 0$ to the linear, power models and gamma distribution of the α and β gamma models. The β_0 and β_1 are parameters related to the model.

In consequence, nine equations were evaluated to estimate millet leaf area. The following criteria were used to evaluate models: Coefficient of model determination (R^2), Akaike's Information Criterion (Akaike,

1974) (AIC), Sum of Square of Residuals (SSR), and the Willmott index (d) (Willmott, 1981).

Let $\hat{Y}_i$ the model-estimated values of leaf area, so the coefficient of model determination is expressed by the ratio between of the model square sum (MSS) and the total sum of squares (SST), that is,

$$R^2 = \frac{MSS}{SST} = 1 - \frac{SSR}{SST} = 1 - \frac{\sum_{i=1}^n (Y_i - \hat{Y}_i)^2}{\sum_{i=1}^n (Y_i - \bar{Y})^2}$$

The Akaike information criteria (AIC), as defined by Akaike (1974), is given by:

$$AIC = -2 \ln L(x|\hat{\theta}) + 2(p)$$

where, $L(x|\hat{\theta})$ is the maximum likelihood function, defined as the production of density function and p is the number of model parameters. The sum of square of the residuals (SSR) is the square sum of difference between the values observed and predicted by the models, where the lowest value contributes to the choice of the best equation. Let $\hat{Y}_i$ the value of the i-th leaf area after model adjustment, then define SSR for this study by following expression:

$$SSR = \sum_{i=1}^n (Y_i - \hat{Y}_i)^2$$

The d index defined by Willmott (1981) is given by:

$$d = 1 - \frac{\sum_{i=1}^n (\hat{Y}_i - Y_i)^2}{\sum_{i=1}^n (|\hat{Y}_i - \bar{Y}| + |Y_i - \bar{Y}|)^2}$$

where, $\bar{Y}$ is the mean of values of leaf area (Y_i). For all analysis and construction of graphics R-project version 3.5.0 software for windows was used.

Results and discussion

The two millet genotype exhibited considerable variation in leaf blade length; computed to length (L) and maximum width (W), product of L x W and real leaf area (RLA) (Table 2).

Leaf blade L for the two millet genotype ranged from 6.80 to 72.50 cm with an average value of 46.07 cm, while W varied between 0.50 to 4.30 cm, with an average value of 2.44 cm. Leaf blade product of L x W showed a maximum value of 288.80 cm² and a minimum value of 3.59 cm²; the average was 124.16 cm². Real leaf area values varied from 209.21 cm² to 3.02 cm², with an average of 95.31 cm². The high level of variation occurred because leaves collected for sampling were from plants at all stages of morphological development. Thus, the equation developed in this research could be applied to all stages and ages of millet development. Pedroso et al. (2009) observed that millet leaf blade length was influenced by intercrop period, varying from 22.73 cm to 30.1 cm. Schmildt et al. (2014) stated that high range values are essential for morphological studies because these provide comparison points when regression models are used for leaf area estimations.

The power regression model had the greatest explanatory capacity, with an R² of 99.96% for the genotype IPA BULK 1BF (Table 3). While the linear and gamma models had R² values of 99.25 and 96.07%, respectively. Furthermore, the power model exhibited a lower sum of squares (5691.25) and AIC (-130.53), and greater Willmott index (0.9911), than the other studied models (Table 3).

For the genotype ADR 300 (Table 4) the linear model showed the highest explanatory power (R²= 99.56%) and highest Willmott index (0.9963) when compared with the other regression models studied.

When we analyzed the combined values for the two millet genotype (Table 5), the linear model showed the greatest explanation power (R² = 99.38%), of all studied regression models. The power model had the lowest SSR (90429.30) and AIC (219.94) when compared to the linear and the gamma models. All three models have similar Willmott index values (Table 5).

Thus, in general, the models that presented the best adjustments agreed with previous studies of leaf area determination in *Urochloa mosambicensis* (Leite et al., 2017), corn (Vieira Junior et al., 2006), passion fruit (Morgado et al., 2015); Mango tree (Lima, Rodrigues, & Lima, 2012), Ginger tree (Kandiannan, Parthasarathy, Krishnamurthy, Thankamani, & Srinivasan, 2009), *Sida cordifolia* and *Sida rhombifolia* (Bianco, Carvalho, & Bianco, 2008), and *Coffea arabica* (Antunes et al., 2008).

Table 2. Mean, standard deviation, median, maximum and minimum values for length (L), width (W), product of leaf blade (L x W) and real leaf area (RLA) per millet genotype.

IPA BULK 1BF genotype					
Variables/units	Mean	standard deviation	Median	maximum	minimum
L (cm)	47.56	14.58	47.50	72.50	12.50
W (cm)	2.40	0.85	2.30	4.10	0.50
L x W (cm ²)	123.25	64.48	121.59	262.70	6.25
RLA (cm ²)	96.51	49.37	92.54	195.71	6.19
ADR 300 genotype					
Variables/units	Mean	standard deviation	Median	maximum	minimum
L (cm)	44.33	17.86	48.30	72.50	6.80
W (cm)	2.48	0.98	2.70	4.30	0.52
L x W (cm ²)	125.21	79.90	128.75	288.80	3.59
RLA (cm ²)	93.91	60.69	104.92	209.21	3.02

Table 3. Estimates of parameters and criteria of adequacy for models considering as the explanatory variable the product between length and width (L x W) for millet genotype IPA BULK 1BF leaf blades.

Models	Equation of real leaf area	Best-Fit Model Criteria			
		R ²	SSR	AIC	D
Linear	$\hat{Y} = 0.7764LW$	99.25	6061.18	508.63	0.9909
Gamma	$\hat{Y} = 0.7947LW$	96.07	6504.19	480.67	0.9905
Power	$\hat{Y} = LW^{0.9492}$	99.96	5691.25	-130.53	0.9911

R²= coefficient of determination; SSR = sum of square of residual; AIC = Akaike information of criteria; d = Willmott of index.

Table 4. Estimates of parameters and criteria of adequacy for models considering the explanatory variable the product between length and width (L x W) for millet genotype ADR 300 leaf blade.

Models	Equation of real leaf area	Best-Fit Model Criteria			
		R ²	SSR	AIC	D
Linear	$\hat{Y} = 0.751LW$	99.56	3157.28	406.25	0.9963
Gamma	$\hat{Y} = 0.754LW$	98.51	3170.43	397.40	0.9962
Power	$\hat{Y} = 0.834LW^{0.976}$	99.00	3463.29	-96.69	0.9958

R²= determination coefficient; SSR= sum of square of residual; AIC= Akaike information of criteria; d= Willmott-of index.

Table 5. Estimates of parameters and criteria of adequacy for models considering the explanatory variable the product between length and width (L x W) for millet leaf blade, independent of genotype.

Models	Equation of real leaf area	Best-Fit Model Criteria			
		R ²	SSR	AIC	D
Linear	$\hat{Y} = 0.764LW$	99.38	9639.21	920.41	0.9936
Gamma	$\hat{Y} = 0.776LW$	97.35	10025.03	882.55	0.9935
Power	$\hat{Y} = 0.879LW^{0.971}$	98.76	9429.30	-219.94	0.9936

R² = determination coefficient; SSR = sum of square of residual; AIC = Akaike information of criteria; d = Willmott-of index.

Comparison of the four criteria (highest R², d index, lowest SSR, and AIC) were used to adjust the model. The best-fit model to explain the real leaf area in the genotype IPA BULK 1BF was the product of length x width (L x W) of the leaf blade with the power model. However, for the ADR 300 genotype the best-fit model was linear. When the genotype was independently analyzed the best-fit model for millet was the power model, because besides exhibiting lowest SSR and AIC values, to it also showed similar Willmott index and R² than the other models studied.

Our results corroborated with those of Leite et al. (2017) who concluded that the best-fit fittest model for estimating foliar area in *Urochloa mosambicensis* is the power model using the product of leaf blade length x width (L x W) as the independent variable. The results of the current study also showed similarities with those of other authors; Cargnelutti Filho et al. (2012) obtained a determination coefficient of 0.992 when estimating *Mucuna pruriens* leaf area using the product of leaf blade length x width (L x W). It is noteworthy that all models studied showed a coefficient of determination (R²) above 0.96, which indicates that variations in leaf area in genotypes of *Pennisetum glaucum* of 96% could be explained by the models used in the present research. Our findings are considered satisfactory for the purpose of this research. Our findings were similar to those reported by Silva et al. (2013) that used the product of length x width (L x W) of leaf blade of the tropical grasses (*Brachiaria brizantha* cv. *Xaráes* and *Panicum maximum* cv. *Massai*). They obtained very accurate estimation

models of leaf area. They also had a coefficient of determination (R^2) of 0.92. Vieira Junior et al. (2006) used similar methodology as our study to test regression models to estimate leaf area in 44 genotypes of corn. They had a coefficient of determination (R^2) which varied between 80.78% to 99.18%.

Figure 1 shows the relation between the real leaf area of genotype IPA BULK 1BF by product of length and width, as well the values adjusted for the models. Note that the adjusted values of all models are similar to observed values, indicating the appropriateness of evaluated models. The same can be seen for the relation of real leaf area of genotype ADR 300 and independent of genotype by the product of length by width, respectively (Figures 2 and 3).

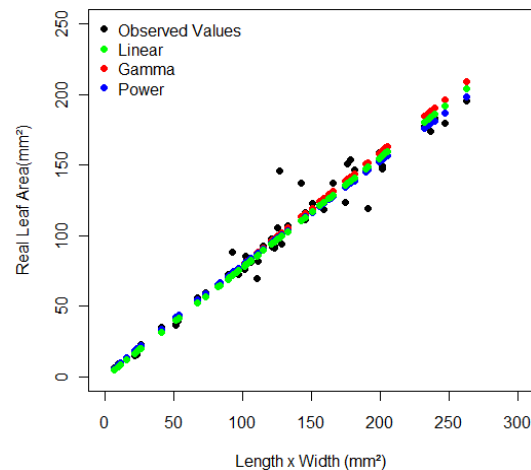


Figure 1. Estimation of real leaf area of the models in relation to the product of length by width for millet leaf blade, genotype IPA BULK 1BF.

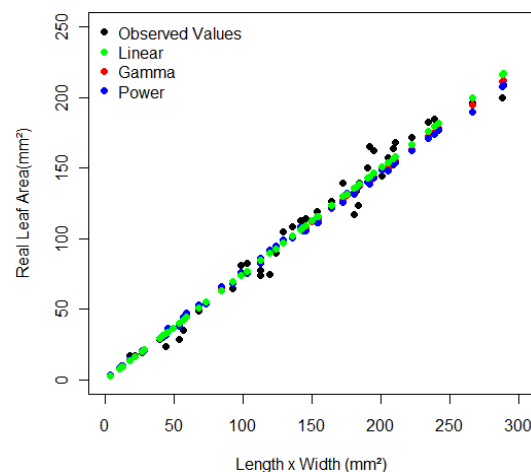


Figure 2. Estimation of real leaf area of the models in relation to the product of length by width of millet leaf blade, genotype ADR 300.

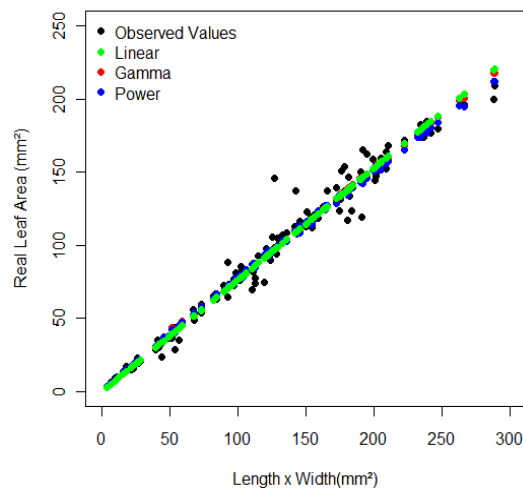


Figure 3. Estimation of real leaf area of the models in relation to the product of length by width of the leaf blade of millet, independent of evaluated genotype.

Conclusion

The product of the length x width (L x W) of the leaf blade is an appropriate parameter for use as an independent variable in regression models, when the aim is to predict-millet leaf area. Independently of the genotype, leaf blade length x width (L x W) used with the power model ($y = 0.879LW^{0.971}$) is the method recommended for determining millet leaf area. Adopting the power model allows estimates the millet leaf area at all ages of plant development, with high accuracy, efficiency, and low cost and without destroying the plant.

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