Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2636
ISSN on-line: 1807-8672
Doi: 10.4025/ actascianimsci.v39i4.36447

Determining a model to estimate leaf area in Pornunça (*Manihot* sp.) using morphometric measures

Guilherme de Lira Sobral Silva[1], Maria Socorro de Souza Carneiro[1], Ricardo Loiola Edvan[2*], Alberício Pereira de Andrade[3], Geovergue Rodrigues de Medeiros[4] and Magno José Duarte Cândido[1]

*[1]Departamento de Zootecnia, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil. [2]Departamento de Zootecnia, Universidade Federal do Piauí, BR 135, km 3, 64900-000, Cidade de Bom Jesus, Piauí, Brasil. [3]Departamento de Zootecnia, Universidade Federal de Pernambuco, Garanhuns, Pernambuco, Brazil. [4]Instituto Nacional Semi-Arido, Campina Grande, Paraíba, Brazil. *Author for correspondence. E-mail: edvan@ufpi.edu.br*

ABSTRACT. Pornunça is a species of the genus *Manihot* that has been cultivated as an option for animal feeding in the Brazilian semi-arid. Leaf area (LA) is important to determine forage plant growth, whose evaluation model has to meet the following characteristics: practicality, precision and low cost. The objective of this study was to set a model to determine the leaf area in Pornunça (*Manihot* sp.) from morphometric measurements of the leaf. The experimental design was in randomized blocks with three methods for determination of leaf area, and three blocks, with 144 replicates (leaves). For the determination of LA, the direct method (FAReal), treadmill leaf area meter (Li-Color 3100©) and linear dimensions were used. Data were subjected to regression analysis. There was a relationship between the actual leaf area (FAReal) for small, medium, large and total leaves, and the leaf area determined by the treadmill leaf area meter (FALi-Cor©) with a high coefficient of determination for linear fit. The Pornunça leaf area obtained by the Li-Cor3100© meter is approximately 10% lower compared to the FAReal method. The model that best fit the Ponunça leaf area estimation was the potential.

Keywords: growth, plant, morphology, semiarid.

Determinação de modelo para estimar área foliar da Pornunça (*Manihot* sp.), utilizando medidas morfométricas

RESUMO. A Pornunça é uma espécie do gênero *Manihot* que está sendo cultivada como opção para alimentação animal no semiárido do Brasil. A área foliar (AF) é importante para determinar o crescimento de planta forrageira, e a avaliação tem que reunir as seguintes características: praticidade, precisão e baixo custo. O estudo objetivou estabelecer um modelo para determinar a área foliar da Pornunça (*Manihot* sp.) a partir de medidas morfométricas da folha. O delineamento experimental foi realizado em blocos ao acaso, com três tratamentos (métodos de determinação de área foliar) e três blocos, com o total de 144 repetições (folhas). Para determinação da AF, utilizou-se o método direto (AFReal), medidor de área foliar tipo esteira (Li-Cor 3100©) por meio das dimensões lineares. Os dados foram submetidos à análise de regressão. Houve uma relação entre a área foliar real (AFReal) para folhas pequenas, médias, grandes e total, e a área foliar determinada pelo medidor de área foliar tipo esteira (AFLi-Cor©) apresentou coeficiente de determinação elevado para o ajuste linear. A área foliar da Pornunça obtida pelo medidor Li-Cor3100© é de aproximadamente 10%, inferior, em comparação ao método AFReal. O modelo que melhor se ajustou para a estimativa da área foliar da Pornunça foi o potencial.

Palavras-chave: crescimento, planta, morfologia, semiárido.

Introduction

Pornunça (*Manihot* sp) is a plant of the family Euphorbiaceae, a direct relative of cassava (*Manihot esculenta*) and maniçoba (*Manihot pseudoglaziovii*) (Silva, Santos, Oliveira, Moraes, & Santana, 2009), which has been grown in the Brazilian northeastern region for animal feed. According to Carvalho et al. (2012), the quality of the *Manihot* species for animal feeding is evidenced by the high protein and non-fiber carbohydrates content in relation to other species commonly used. In this way, the rational exploitation of Pornunça shoots can be an important alternative for feeding ruminants in this region.

For studies on the growth of plants, it is necessary to know the characteristics of growth and development. Among these characteristics, leaf area (LA) is an important parameter (Lima, Oliveira, Medeiros, Oliveira, & Oliveira, 2008). The measurement of leaf area is important in studies related to morphology, anatomy and ecophysiology (Moraes, Santos, Wisser, & Krupek, 2013).

The estimation of leaf area can be carried out by measuring instruments (destructive methods) or by regression models (non-destructive methods) according to Francisco, Diotto, Folegatti, Silva and Piedade (2014). There are several methods to measure the leaf area with good precision, being classified as destructive and non-destructive, direct or indirect according to Marshall (1968).

Among the methods most used to determine LA, one of the most requested are: the destructive method, where the leaves are collected and taken for laboratory analysis where the treadmill leaf area meter (LI-COR 3100©) is installed. This method estimates the area by the principle of grid cells of known area (LI-COR Biosciences, 1996). The destructive method demands time and labor, another disadvantage in the evaluation of leaf area is the necessity of a large number of plants in the plot for quantification at different times of the cultivation period (Lima et al., 2008), besides the high cost in the purchase of the device.

The non-destructive method is also widely used by researchers; in this, the estimation of leaf area is performed through mathematical equations as presented by Moraes et al. (2013), which consist of the relationship between the length of the central rib (C) and the maximum width (L) and the relationship between these measures (CxL). The financial limitation for acquisition of apparatus for measuring leaf area makes mathematical models an important tool in the scientific context. According to Pinto, Andrade, Pereira, Arruda and Andrade (2007), studies on the correlation of leaf area from leaf blade length and width measurements have generated equations with excellent estimation accuracy in relation to more sophisticated and expensive methods.

There are numerous researches to determine the leaf area in several annual and perennial crops however this index is poorly studied in crops of the genus *Manihot*, especially for Pornunça. Therefore, understanding the growth and development of this species can allow the practice and management strategies that raise the productivity of this important forage resource for the northeast region of Brazil. Thus, this study was carried out with the goal of determining the model to estimate the leaf area of Pornunça (*Manihot* sp.) from morphometric measurements of leaf blade.

Material and methods

The study was conducted from May 2010 to October 2011, at the Experimental Station of the National Semi-Arid Institute (INSA), located in the municipality of Campina Grande, State of Paraíba, Brazil, at the geographical coordinates 7°16'35.9"S and 35°57'55.3"W with 552 m altitude, maximum temperature of 31-32°C and minimum around 23-15°C, relative air humidity between 75-82% and rainfall of 1,046 mm^3.

This was a randomized block experimental design with three treatments (methods of determination of leaf area), with three blocks, each block with 16 experimental units, totaling 48 plants, being evaluated three leaves per plant, with a total of 144 leaves (repetition). The experimental units had an area of 6 x 7 m, resulting in plots of 102 m^2. The soil was prepared in a conventional way by plowing and harrowing before planting. The soil was collected at horizon A, at a depth of 20 cm. The soil presented a textural classification Areia Franca (Natural Resources Conservation Service [NRCS], 2016) with sandy texture (820 g kg^{-1} sand, 80 g kg^{-1} silt and 100 g kg^{-1} clay) and the following chemical properties: pH ($CaCl_2$ 0.01M) = 4.3; OM = 9.4 g dm^{-3}; P = 2.9 mg dm^{-3}; K = 0.17 $cmol_c$ dm^{-3}; Ca = 1.4 $cmol_c$ dm^{-3}; Mg = 0.8 $cmol_c$ dm^{-3}; Na= 0.69 $cmol_c$ dm^{-3}; H+Al = 3.10 $cmol_c$ dm^{-3}; T = 6.2 $cmol_c$ dm^{-3} and V(%) = 49.7.

The area was planted with Pornunça seedlings obtained from cuttings collected in the region, planted in plastic bags containing vegetable sand substrate and tanned bovine manure, in a ratio of 2: 1. Then, seedlings were transplanted (May 2, 2010) to pits containing 6 liters of bovine manure tanned in spacings of 2.0 x 2.0m; after 45 days, a uniformity cut was made at a height of 50 cm. Previously, the soil was fertilized with nitrogen, phosphorus and potassium according to the soil analysis, and considering the cassava crop, it was not necessary to make the soil correction. Nitrogen levels (12.75 g $plant^{-1}$ urea at the bottom and 17 g $plant^{-1}$ urea as topdressing), phosphorus (56 g $plant^{-1}$ single superphosphate at the bottom) and potassium (11.30 g $plant^{-1}$ potassium chloride at the bottom) were applied, as described.

At random, 48 plants were duly identified with colored wire to monitor vegetative growth. At the end of the experimental period (463 days after the uniformity cut), the plants were cut at 50 cm from the ground, placed individually in plastic bags and transported to Campina Grande Federal University, Campina Grande Campus, State of Paraíba, Brazil. Then, 3 leaves per plant were collected, in a total of

144, to determine the leaf area (LA) using the direct method (AFReal). In the direct method, transparency sheet was used to outline the leaf. The leaf outlines were transferred to A4 paper from the same ream, then cut and weighed on an analytical balance, recording all decimal places to minimize the margin of error. The selected leaves did not present any type of damage or attack of disease or pest and were at full stage of vegetative development. Next, a square of paper of the same origin as the previous drawings, measuring 10 cm x 10 cm, equivalent to 100 cm^2, weighing 0.810 g was cut out.

For determination of AFReal, we used the same 144 leaves to measure the LA by means of a treadmill leaf area meter (Li-Color 3100©). In the analysis of the data, LA was classified in three size classes: ranging from 7.00-52.90 cm^2 small leaves; 53.00-86.90 cm^2 medium leaves; and 87.00-202.00 cm^2 large leaves. This classification was carried out in order to verify a possible variation in the calculation of LA, as a function of leaf size and method for determination of leaf area.

In the same 144 leaves, we also measured the L-width (distance between the apex of the first two leaflets) and the C-length (distance between the base of the leaf and the apex of the central leaflet) using a 50 cm ruler, aiming to find a relationship between these variables and the actual leaf area. All measures were taken following recommendations of Benincasa (1988).

The obtained data were subjected to regression analysis to obtain linear $y = bx$ and exponential $y = e^{bx}$ equations, where the value of 'b' obtained corresponds to the correction coefficient of the model. The equations were obtained by Excel 2007© software.

Results and discussion

There was a relationship (Figure 1A, B and C) between the actual leaf area (AFReal) for small, medium, large and total leaves, and the leaf area determined by the treadmill leaf area meter (AFLi-Cor) with a high coefficient of determination for the linear fit. According to Francisco et al. (2014), a high coefficient of determination indicates a close relationship between the leaf area and the linear measurements of the plants.

For small leaves (Figure 1A), in the relationship between the two methods, the dispersion of data is larger for medium and large leaves (Figure 1B and C). In this case, the leaf area determined by AFLi-Cor is about 20.62% smaller than that obtained by AFReal. The linear equation passing through the origin has an angular coefficient of 0.79, therefore less than 1. However, as leaf size increased, the points between these two methods became equivalent, with values of 10.35% for medium leaves and 9.49% for large leaves (Figure 1B and C).

Probably, the difference between the two methods (AFLi-Color and AFReal) may be related to the type of leaf of Pornunça that did not fit properly to the treadmill meter. At the moment of leaf displacement errors may have occurred due to the morphological sinuosity of the leaf, thus the leaf microelevations make difficult the scanner reading in the methods. In medium and large leaves, the effect of leaf sinuosity tends to reduce, because they are larger and adjust better on the treadmill, being more horizontal at the moment of the passage in the equipment. On the other hand, the AFReal method relies exactly on the reproduction of the original shape (drawing) of the paper, which reduces possible errors due to the leaf morphology, regardless of the size and microelevations of the leaves.

The selection of the method for estimating leaf area depends, among others, on the degree of precision required, sample size and leaf morphology. The most precise methods are the destructive ones, with the disadvantage of impeding the continuity of the studies in the same plant (Coelho, Angelocci, Vasconcelos, & Coelho, 2005). However, when using all the leaves (Figure 1D), regardless of the size class, the angular coefficient of the line approaches 1, but the leaf area determined by the treadmill leaf area meter (AFLi-Cor), is still approximately 10% lower than the direct method (AFReal).

In relation to the coefficient of determination (R^2) of the obtained equations, the AFLi-Cor and AFReal relationship (Figure 1A, B and C) presented satisfactory fits of the points to the line, with determination coefficient of 0.79; for small leaf, 0.84 for medium leaf and 0.93 for large leaf when classified by leaf size and 0.97 when disregarding leaf classification (Figure 1D). According to Lucena, Batista, Dombroski, Lopes and Rodrigues (2011), the model is suitable when it presents high coefficient of determination with the reference standard.

According to the results in Figure 1, it is possible that the separation of leaves into size class can contribute to maximize the coefficient of determination (R^2) when leaves are grouped into a single size class. According to Silva, Leite and Ferreira (2008), the visual classification of the leaves in groups of different sizes contributes to the reduction of the total number of leaves necessary for the estimation of the leaf area in routine studies, without compromising the evaluation, as well as to reduce the variance in the obtained data, due to the homogeneity of the leaf areas.

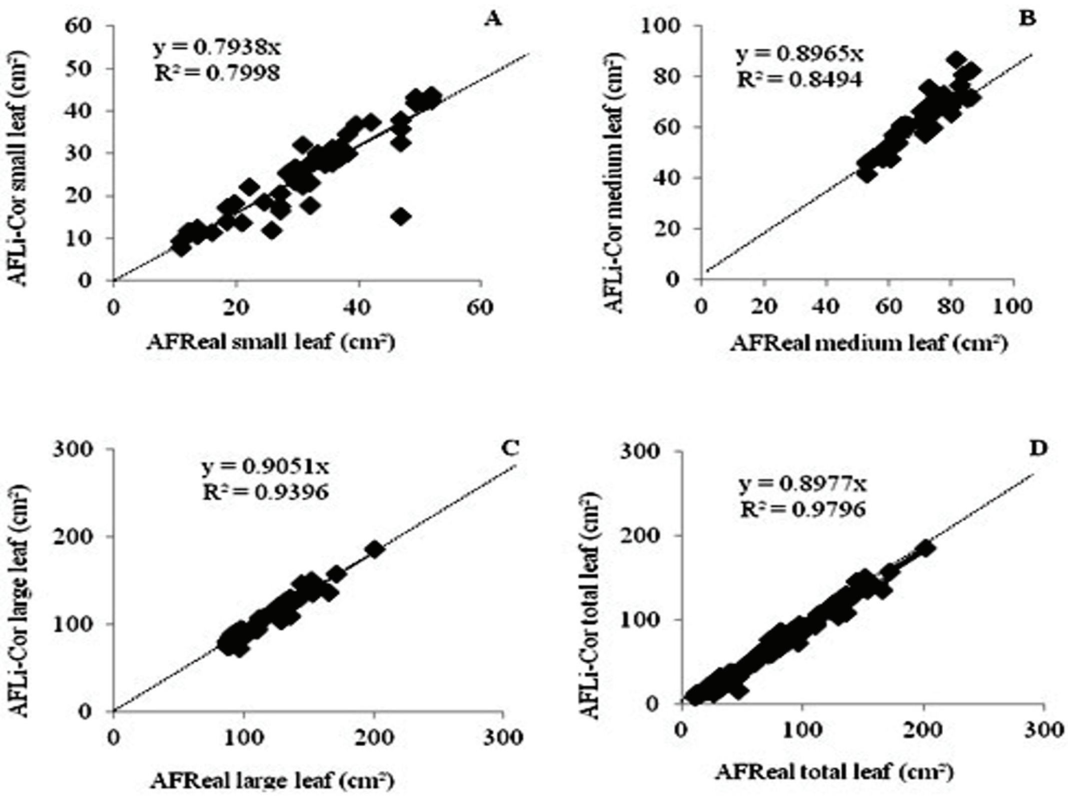

Figure 1. Relationship between the direct method (AFReal) and the treadmill leaf area meter (AFLi-Cor) for small leaf (A), medium leaf (B), large leaf (C) and total leaf (D) of Pornunça (*Manihot* sp.).

The results in Figure 1 demonstrate that in the AFReal method, the LA points most closely approximate the obtained line for the 1: 1 ratio.

It can be seen in Figures 2 and 3 that among the mathematical models tested for length, width, and product between these two leaf variables, the potential-type equations presented better fit to the curve. It should be noted that in the potential model the value of the coefficient of determination was high for both AFLi-Cor and AFReal, when the product is applied between the length and width of the leaf (C*L) highlighted by the excellent fits, with R^2 of 0.94 and 0.96, respectively. This is corroborated by Pinto et al. (2007), who observed a high correlation between AFReal and the product of leaf length by width for estimating maniçoba LA.

When correlating the C*L with the AFReal method to estimate leaf area (Figure 3C), there was a coefficient of determination (R^2 = 0.96) higher than that analyzed separately (Figures 2A and B, Figure 3A and B) and with the same relationship using the AFLi-Color method, demonstrating that the method provides a high precision in determining the leaf area for this species, as it presents an excellent fit of the points to the curve, where 96% of the observed variations in leaf area were explained by the equation. Francisco et al. (2014) observed that the regression between the leaf area and the product of the linear dimensions (CxL) of the pineapple leaves allowed a better fit of the linear model.

A similar observation was made by Lima et al. (2008), in the study on mathematical models to estimate the leaf area of cowpea (R^2 = 0.95), a result higher than that reported by Pinto et al. (2007) for two maniçoba species (R^2 = 0.89). This is consistent with Monteiro et al. (2005), who studied regression analyses of the leaf area with the leaf length and width, separately, and showed lower levels of correlation than the one run with the product of the two dimensions.

Nevertheless, for the common bean (Queiroga, Romano, Souza, & Miglioranza, 2003), for sunflower (Maldaner et al., 2009) and for *Chrysobalanus icaco* (Cunha et al., 2010), the authors did not obtain satisfactory results when used the product C*L of the leaf. In other crops, the leaf development and shape may explain the low adjustments to the leaf C*L product than those generated with only one of the linear dimensions.

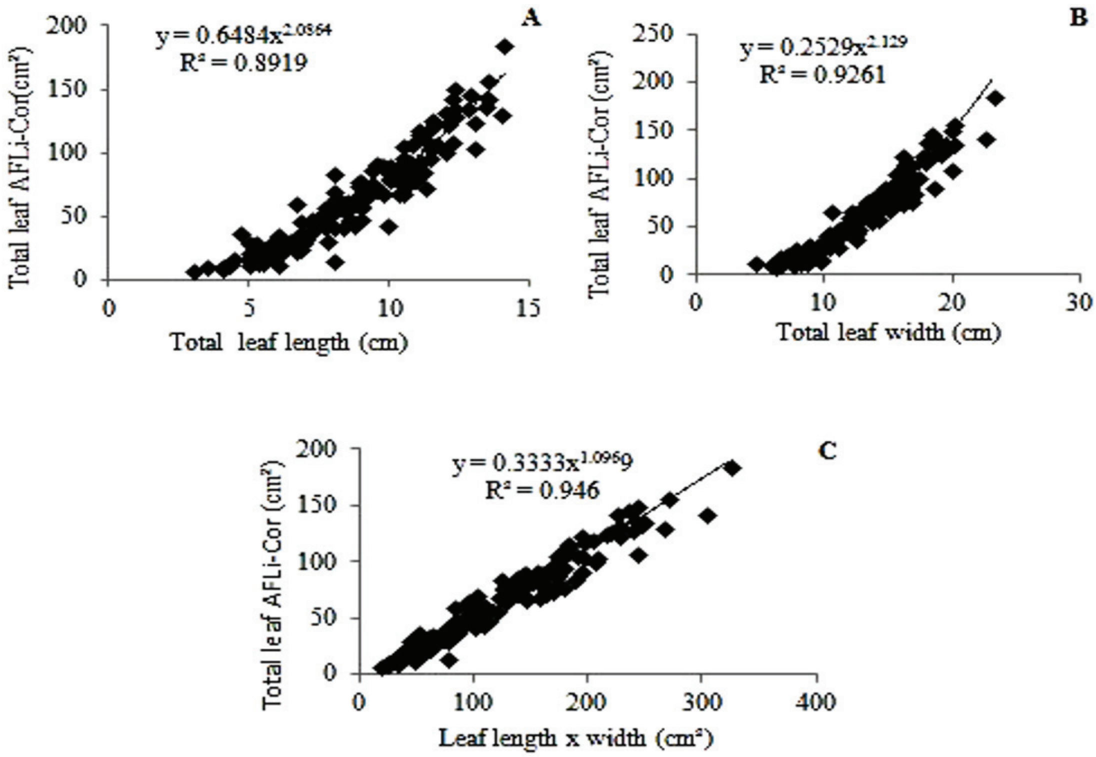

Figure 2. Relationship between total leaf length (A), total leaf width (B) and total leaf length x width (C) by treadmill leaf area meter (AFLi-Color) in Pornunça (*Manihot* sp.).

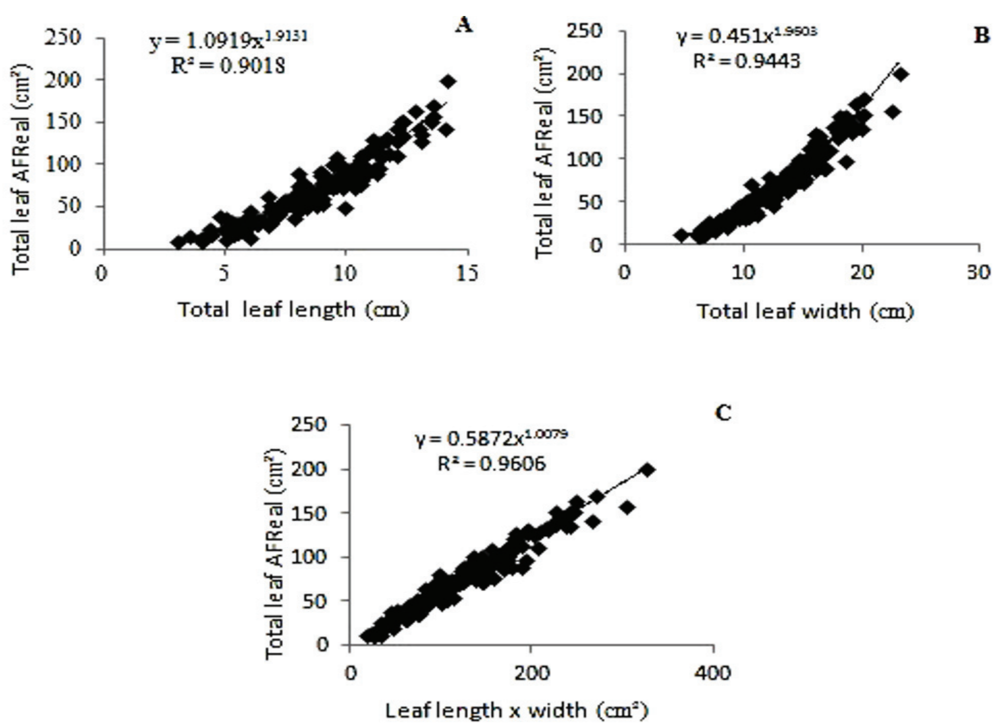

Figure 3. Relationship between total leaf length (A), total leaf width (B) and total leaf length x width (C) by direct method (AFReal) in Pornunça (*Manihot* sp.).

Even with some dispersion of data in relation to the curve, it is suggested that the equations represent excellent fit for leaf area estimation. In addition to presenting the following advantages in relation to the treadmill leaf area meter (AFLi-Color): lower cost (acquisition and maintenance of the apparatus) in obtaining the results, practicality and it is not a destructive method.

Conclusion

The leaf area of Pornunça determined by the Li-Cor 3100 treadmill leaf area meter is lower than by the direct method. The difference between these methods increases as the leaf sizes decrease.

The estimation of the leaf area from the morphological dimensions is closer to the actual leaf area when used the product of leaf width and leaf length, regardless of its size. The model that best fit the leaf area estimation was the potential.

References

Benincasa, M. M. P. (1988). *Análise do crescimento de plantas: noções básicas* (Boletim Técnico, 467). Jaboticabal, SP: FUNEP.

Carvalho, P. L. P. F., Silva, R. L., Botelho, R. M., Damasceno, F, M., Rocha, M. K. H. R., & Pezzato, L. E. (2012). Valor nutritivo da raiz e folhas da mandioca para a tilápia do Nilo. *Boletim do Instituto de Pesca, 38*(1), 61-69.

Coelho Filho, M. A., Angelocci, L. R., Vasconcelos, M. R. B., & Coelho, E. F. (2005). Estimativa da área foliar de plantas de lima ácida 'Tahiti' usando métodos não-destrutivos. *Revista Brasileira de Fruticultura, 27*(1), 163-167.

Cunha, J. L. X. L., Nascimento, P. G. M. L., Mesquita, H. C., Silva, M. G. O., Dromboski, J. L. D., & Silva, I. N. (2010). Comparação de métodos de área foliar em *Chrysobalanus icaco* L. *Revista Agropecuária Científica no Semi-Árido, 6*(3), 22-27.

Francisco, J. P., Diotto, A. V., Folegatti, M. V., Silva, L. D. B., & Piedade, S. M. S. (2014). Estimativa da área foliar do abacaxizeiro cv. Vitória por meio de relações alométricas. *Revista Brasileira de Fruticultura, 36*(2), 285-293.

LI-COR Biosciences. (1996). *LI 3100 area meter instruction manual.* Lincoln, NE: LI-COR.

Lima, C. J. G. S., Oliveira, F. A., Medeiros, J. F., Oliveira, M. K. T., & Oliveira Filho, J. F. (2008). Modelos matemáticos para estimativa de área foliar de feijão caupi. *Revista Caatinga, 21*(1), 120-127.

Lucena, R. F., Batista, T. M., Dombroski, J. L., Lopes, W. A., & Rodrigues, G. S. (2011). Medição de área foliar de aceroleira. *Revista Caatinga, 24*(2), 40-45.

Maldaner, I. C., Heldwein, A. B., Loose, L. H., Lucas, D. D. P., Guse, F. I., & Bortoluzzi, M. P. (2009). Modelos de determinação não-destrutiva da área foliar em girassol. *Ciência Rural, 39*(5), 1356-1361.

Marshall, J. K. (1968). Methods of leaf area measurement of large and small leaf samples. *Photosynthetica, 2*(1), 41-47.

Monteiro, J. E. B. A., Sentelhas, P. C., Chiavegato, E. J., Guiselini, C., Santiago, A. V., & Prela, A. (2005). Estimação da área foliar do algodoeiro por meio de dimensões e massa das folhas. *Bragantia, 64*(1), 15-24.

Moraes, L., Santos, R. K., Wisser, T. Z., & Krupek, R. A. (2013). Avaliação da área foliar a partir de medidas lineares simples de cinco espécies vegetais sob diferentes condições de luminosidade. *Revista Brasileira de Biociência, 11*(4), 381-387.

Natural Resources Conservation Service [NRCS]. (2016). *Soil mechanics level I: Module 3.* USDA Textural Soil Classification. National Water and Climate Center. Recovered from http://www.wcc.nrcs.usda.gov

Pinto, M. S. C., Andrade, A. P., Pereira, W. E., Arruda, F. P., & Andrade, M. V. M. (2007). Modelo para estimativa da área foliar da maniçoba. *Revista Ciência Agronômica, 38*(4), 391-395.

Queiroga, J. L., Romano, E. D. U., Souza, J. R. P., & Miglioranza, E. (2003). Estimativa da área foliar do feijão-vagem *(Phaseolus vulgaris* L.) por meio da largura máxima do folíolo central. *Horticultura Brasileira, 21*(1), 64-68.

Silva, A. R., Leite, M. T., & Ferreira, M. C. (2008). Estimativa da área foliar e capacidade de retenção de calda fitossanitária em cafeeiro. *Bioscience Journal, 24*(3), 66-73.

Silva, A. F., Santos, A. P. G., Oliveira, A. P. V., Moraes, S. A., & Santana, L. M. (2009). Produção de forragem e composição química da pornunça cultivada sob solo com fertilidade natural em Petrolina-PE. *Revista Brasileira de Agroecologia, 4*(1), 1492-1496.

Received on March 27, 2017.
Accepted on May 10, 2017.