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Comparison of image analysis softwares for the determination of leaf area.

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A B S T R A C T

Leaf area estimation is a fundamental component for plant development models, since it allows to understand the ecophysiological adaptations to environmental stimulus or management practices. In this sense, we aimed to evaluate the accuracy of three image analysis software that measures area (LA), length (L) and leaf width (W), respectively, supplying the lack of guidance regarding the validation and comparison of these software in biological allometric studies. A total of 176 elliptical leaves were randomly selected, which, after being scanned, were measured using ImageJ, Image-Pro® PLUS and AFSoft software. The LA, L and W foliar measured by each software were submitted to the Kolmovorov-Smirnov and Bartlett tests for confirmation of normality and homoscedasticity, respectively. The ANOVA was performed considering the three softwares for the leaf area. For the leaf length and width, the Student t-test between ImageJ and Image-Pro® PLUS was used. In the latter, additionally by means of an exponential model $LA = \beta_0*(L*W)^{\beta 1}$, the residues were compared. Our results showed that the software ImageJ, Image-Pro® PLUS and AFSoft did not show significant differences in the measurement of leaf area in ANOVA (F= 1.581; p-value = 0.267). Comparing the measurement of leaf width and length, ImageJ and Image-Pro® PLUS software did not show significant differences with the Student t-test (t = -0.248; p-value = 0.804 and t = -0.812; p-value= 0.417, respectively). Likewise, the residues of the exponential model did not show significant differences between them (t = -0.027; p-value= 0.979). Therefore, we conclude that it is possible to determine leaf area and leaf dimensions with the same accuracy using free software such as ImageJ and AFSoft, so that students and researchers should not limit themselves to using paid software for fear of losing accuracy.

Keywords: ImageJ, Image-Pro® PLUS, AFSoft, Allometric model

Comparação de softwares de análise de imagem para a determinação da área foliar.

R E S U M O

A estimativa da área foliar é um componente fundamental para os modelos de desenvolvimento das plantas, pois permite compreender as adaptações ecofisiológicas perante estímulos do meio ambiente ou de práticas de manejo. Nesse sentido, objetivamos avaliar a acurácia de três softwares de análise de imagem que mensuram a área (AF), comprimento (C) e largura (L) foliar, respectivamente, suprindo a falta de direcionamento quanto à validação e comparação destes softwares em estudos biológicos alométricos. Foram selecionadas aleatoriamente 176 folhas elípticas, as quais depois de digitalizadas, foram mensuradas com os softwares ImageJ, Image-Pro® PLUS e AFSoft. A AF, C e L foliar mensurados por cada software foram submetidos aos testes de Kolmovorov-Smirnov e Bartlett para confirmação da normalidade e homocedasticidade, respectivamente. A ANOVA foi realizada considerando os três softwares para a área foliar. Para o comprimento e largura foliar foi utilizado o teste t-Student entre ImageJ e Image-Pro® PLUS. Nestes últimos, adicionalmente por meio de um modelo exponencial $AF= \beta_0*(C*L)^{\beta 1}$, foram comparados os resíduos. Nossos resultados evidenciaram que os softwares ImageJ, Image-Pro® PLUS e AFSoft não demonstraram diferenças significativas na mensuração da área foliar na ANOVA (F=1,581; p-valor=0,267). Comparando a mensuração da largura e comprimento foliar, os softwares ImageJ e Image-Pro® PLUS não apresentaram diferenças significativas com o teste t-Student (*t* = -0,248; p-valor= 0,804 e *t* = -0,812; p-valor= 0,417, respectivamente). Da mesma forma, os resíduos do modelo exponencial tampouco apresentaram diferenças significativas entre si (*t* = -0,027; p-valor= 0,979). Portanto, concluímos que é possível determinar com a mesma exatidão a área foliar e dimensões foliares fazendo uso de softwares livres como ImageJ e AFSoft, de modo que os estudantes e pesquisadores não deveriam se limitar à utilização de softwares pagos por receio de perder acurácia.

Palavras-Chaves: ImageJ, Image-Pro® PLUS, AFSoft, Modelo alométrico

1. Introduction

The leaves are the main structures of the plants that participate in the photosynthetic process (SEVERINO; CARDOSO; SANTOS, 2005). Studies on foliar dimensions, such as leaf area (LA), are useful in estimating the physiological and nutritional needs of a crop, which consequently influence the development and productivity of the plants (ALLEN et al., 1997; TERUEL, 1995). In ecological studies, interest in LA is widely disseminated to investigate processes of adaptation, evolution, competition, and composition of traits in plant assemblages (PÉREZ-HARGUINDEGUY et al., 2013; WRIGHT et al., 2004). In addition, the LA is important for the excellent role in studies that consider the relation between plant-environment (TRAISER et al., 2005; XU et al., 2009), and functional diversity in local and biogeographic gradients (DÍAZ et al., 2015; MUSCARELLA; URIARTE, 2016; SFAIR; ROSADO; TABARELLI, 2016), and for the reconstruction of paleobotanical landscapes (ROYER et al., 2005). In agronomic studies the leaf is considered a key variable, due to the high susceptibility to climate change (WRIGHT et al., 2004) and the relevance to vital functions of plants such as evapotranspiration, interception and light absorption. In addition, LA is considered an indispensable parameter for the calculation of the specific leaf area (ratio between the dry leaf mass ratio and its area), globally recognized as a "hotspot feature" in plant ecology (DÍAZ et al. 2015).

Determination of LA can be performed by direct or indirect methods. In the field, direct or destructive measurements require time and usually require the use of expensive equipment, such as portable meters (MALDANER et al., 2009). In addition, the leaves are removed from the plant and measured by digital apparatus that demand high cost. For example, the gravimetric and weighing method of the leaves discs may represent disadvantages in terms of the measurement time limit, which may induce experimental errors related to the destructive effects of the leaves (NASCIMENTO et al., 2002; TAVARES-JÚNIOR et al., 2002). On the other hand, the indirect or non-destructive methods, ie. based on the linear measurement of the leaves of the plants, are associated to the use of allometric models that, when available, allow the determination of the leaf area in situ conditions, facilitating the growth monitoring without the take of the leaves from the plants (ANTUNES et al., 2008; POMPELLI et al., 2012). The last a methodology has become an alternative for researchers interested in predicting LA in various plant groups.

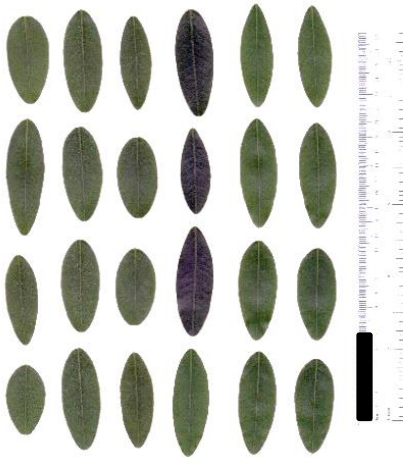
The allometric models are associated to the elaboration of reliable mathematical equations for leaf area estimation, reducing costs in the use of equipment and human resources (KHAN; ZAKI; ABBAS, 2015). However, for its elaboration is necessary a sampling of leaves of different sizes, which are digitalized and finally measured with image analysis software (ANTUNES et al., 2008; POMPELLI et al., 2012; SANTOS, 2016). Among the various image analysis software, Image-Pro® PLUS stands out as a reference software commonly used in studies related to the determination of cellular dimensions, as well as the foliar anatomy of plants (MANTUANO; BARROS; SCARANO, 2006; PITA -BARBOSA et al., 2009). However, because it is a software with original paid license, it becomes difficult to access to students and researchers interested in proposing allometric models in plants. But, there are softwares that are available in free version such us ImageJ (FERREIRA; RASBAND, 2012) and AFSoft (JORGE; SILVA, 2009) that can help in these kinds of works. However, until now exists resistance on the part of researchers regarding the use these softwares for loss accuracy in their studies. In this sense, the present work aimed to compare the accuracy of the use of three image analysis software: ImageJ, Image-Pro® PLUS and AFSoft, to measure leaf area as well as length and width dimensions.

2. Materials and Methods

To determine the leaf area (LA), 176 elliptical leaves were collected randomly, in good condition, without evidence of fungal or insect contamination, in the city of Recife, state of Pernambuco-PE, Brazil. After the collection, the leaves were scanned at a resolution of 300 dpi with common scanner, considering the requirements of each software. For ImageJ and Image-Pro® PLUS softwares, the leaves were scanned together,

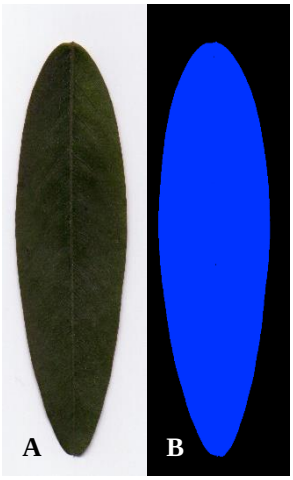
arranged parallel to the main vein, so that the software recognizes the length and leaf width automatically (Figure 1).

Figure 1 Set of scanned leaves for measuring leaf dimensions with ImageJ and Image-Pro® PLUS. Bar: 3 cm.



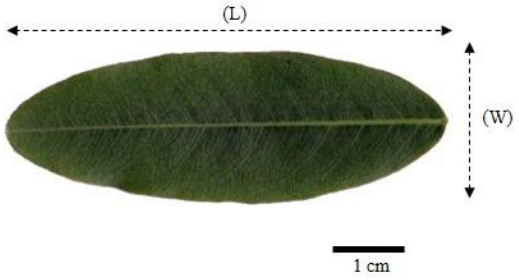
For the AFSoft software, the leaves were scanned individually, since the image processing is based on neural networks to classify the patterns based in the color contrasts of the leaves (Figure 2). This software, even analyzing the leaves individually, can perform the process in batches guaranteeing fastness to the procedure.

Figure 2 Individual leaves scanned before (A) and then (B) analyzed by AFSoft software.



After scanned, the leaf areas were measured by the three image softwares, ImageJ, Image-Pro® PLUS and AFSoft. However, for measurement of length (L) and maximum width (W), respectively, only ImageJ and Image-Pro® PLUS were used. Leaf dimension data were submitted to the Kolmovorov-Smirnov and Bartlett tests for confirmation of normality and homoscedasticity, respectively. The ANOVA was performed to determine significant differences between measurements of leaves leaf areas by the three softwares. The Student's t-test was used to compare leaf length and width, as well as the calculated residuals of the exponential model $LA = \beta_0*(L*W)^{\beta 1}$ (ANTUNES et al., 2008) for ImageJ and Image-Pro® PLUS software. The statistical analyzes were developed using Software R v. 3.5.0 (R CORE TEAM, 2018).

Figure 3 Schematic of the measurements on the leaf blade, with "L" as length and "W" the maximum width, respectively.



In addition, we group the main characteristics of the software and consult in academic search sites the frequency of use in scientific works.

3. Results

3.1 S*oftwares comparison*

The foliar dimensions measured by the three softwares under study are grouped in Table 1. AFSoft was the only software that could not measure the width and length of the leaves. In this sense, the statistical comparison of the three software was performed only for the leaf area, and the leaf length and width for ImageJ and Image-Pro® PLUS.

Table 1 Statistical summary of leaf dimensions measured by the softwares. Mean ± SD, *n* = 176.

Foliar dimensions	ImageJ	Image-Pro® PLUS	AFSoft
Area (cm²)	14.03 ± 3.25	14.64 ± 3.38	14.21 ± 3.29
Width (cm)	2.50 ± 0.32	2.51 ± 0.30	-
Length (cm)	7.42 ± 1.17	7.53 ± 3.38	-

Using the analysis of variance (ANOVA), the leaf area did not show significant differences between Image-Pro® PLUS, ImageJ and AFSoft software (*P*> 0.05) (Table 2).

Table 2 Analysis of Variance (ANOVA) for the leaf area measured by the three image softwares.

FV	GL	SQ	QM	F	[1]p-valor
Softwares	2	34.6	17.29	15.811	0.2067
Erro	525	5741.3	10.936		

[1] If p-valor > 0.05, then there are not significant differences.

On the other hand, when comparing the leaf length and width dimensions, as well as the residues obtained from the exponential model $LA = \beta_0*(L*W)^{\beta 1}$, there were no significant differences (*P*> 0.05) for ImageJ and Image-Pro® PLUS (Table 3).

Table 3 Student's t-test for leaf length and width, as well as for residues obtained from foliar dimensions in the exponential model $LA = \beta_0*(L*W)^{\beta 1}$, measured by ImageJ and Image-Pro® PLUS software.

Dimensions/parameters	Statistical (*t*)	[1]p-valor
Width	-0.248	0.804
Length	-0.804	0.417
Residues obtained from the allometric model $LA= \beta_0*(L*W)^{\beta 1}$	-0.027	0.979

[1] If p-valor > 0.05, then there are not significant differences.

3.2 Main features of the softwares

According to the informative survey carried out (Table 4), we identified that Image-Pro® PLUS represents 94% of the references and searches reported, which confirms the high degree of reputation of the software associated with its various uses in scientific works.

Table 4 Comparison of the general aspects of image analysis software, ImageJ, Image-Pro® PLUS and AFSoft for basic measurements of leaf dimensions.

Aspectos	ImageJ	Image-Pro® PLUS	AFSoft
Google Scholar	171 000	2 040 000	46
Crossref	646	585 310	0
Price	Free	Previous budget	Free
License	Free	Institutional	Free
Technical support	Yes	Yes	Yes
Management	Auto and semi-automatic	Auto and semi-automatic	Automatic

Foliar dimensions	Area, width, length and perimeter	Area, width and length	Area
Excel export	Yes	Yes	Yes
Usage	Low	Hight	Intermediate
Formats	*.gif, *.jpg, *.bmp, *.png, *.pgm, *.fits	*.flf ,.fts, *.fit, *.fits, *.jpg, *.jpg, *.jpf,*.raw ,*.tif, *.tiff	*jpg
Sites	imagej.nih.gov/ij/	www.mediacy.com/imagepproplus	www.cnpdia.embrapa.br/downloads/afsoft/
Systems	Windows®, Mac OS X, Linux	Windows®	Windows®

4. Discussion

Based on the results presented on comparison of the software for image analysis, it was verified that there is a possibility of choosing an intuitive and simple software without problem for the students or researchers, and not worry about that lead them to focus on manipulation errors (SALAS, 2008). The choice of a software often depends on the training of the user, as well as the purpose, either for teaching or research (SOUSA, SILVA, 2000). In this sense, from the statistical point of view with the results of this work, users would have the option to choose any of these free software, since no statistical differences were evidenced among themselves, not only because of the measured leaf dimensions, but because when these dimensions were submitted to the exponential model $LA = \beta_0*(L*W)^{\beta 1}$ (ANTUNES et al., 2008), with focus on mathematical residues, they did not present differences.

Only two study software, ImageJ and Image-Pro® PLUS, proved to be more complete when measuring all leaf dimensions. AFSoft was only able to measure leaf area due to its neural network system that contrasts the images color patterns (JORGE; SILVA, 2009). In addition, for this software, it is necessary that the leaves are scanned individually, which prolongs the execution time of the procedure.

Considering the practicality and compatibility with the user, ImageJ stands out for its easy installation and handling. As a free software, it facilitates access to any user, allowing students or researchers to generate information without restrictions. ImageJ also allows integration with the R software environment (KATABUCHI, 2015), which would provide even more control in the data processing.

The use of free software is a continuous trend that has been advancing over the years, since it allows the user to execute, copy, share, modify and even improve software according to their needs and expectations (SILVEIRA, 2003). On the other hand, it also allows to reduce the illegal practices of use of softwares that need license. Considering that control of the use of software with legal licenses in Latin America is limited, the use of unlicensed software is a commonly accepted practice, with an average incidence of 55% in South America, being 47% in Brazil (BUSINNES SOFTWARE ALLIANCE, 2016).

Due to the justification of high prices for used programs, including at university level, there is an incentive to use unlicensed software, which is distributed to students for individual use. However, in this way, it is difficult to reduce piracy because in a lot of cases it is through teaching where is transmits the knowledge

and ethical-moral principles in the use of illegal softwares. In this sense, the use of unlicensed programs should not happen in the academic community (FERES, MARCOS VINÍCIO CHEIN OLIVEIRA, JORDAN VINÍCIUS DE and GONCALVES, 2017; SALAS, 2008).

5. Conclusion

The use of free software for the evaluation of leaf dimensions on plants such as ImageJ and AFSoft, tend to be fast and easy and do not show a decrease in accuracy compared to Image-Pro® PLUS software. With these free softwares you can carry out quality research, with less cost and accuracy equivalent to Image-Pro® PLUS. ImageJ is a free license, complete and easy-to-use software that automates the measurement of foliar dimensions, helping to produce allometric models.

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