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Full Length Research Paper

Scanner image methodology (SIM) to measure dimensions of leaves for agronomical applications

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A scanner image methodology was used to determine plant dimensions, such as leaf area, length and width. The values obtained using SIM were compared with those recorded by the LI-COR leaf area meter. Bias, linearity, reproducibility and repeatability (R&R) were evaluated for SIM. Different groups of leaves were scanned and measured. R&R studies showed that, the lowest SIM's resolution was nineteen categories. SIM's R&R ANOVA showed the method's measurement error was not significant. In the image processing method, the color image was converted to gray scale over the green band and it was segmented using Otsu methodology. The noise produced was cleaned with a median filter. The leaf image was rotated to align the longest parallel line to y-axis or x-axis using central moments. From the centroid using directional erosions the leaf width and length were obtained and recorded.

Key words: Leaf area, width, length, digital image analysis, segmentation.

INTRODUCTION

Leaf area is an important variable for most physiological and agronomic studies involving plant growth, light interception, photosynthetic efficiency, evapotranspiration and response to fertilizers, irrigation and even vegetal taxonomy. Vegetable crops are very sensitive to environmental conditions which generally affect leaf area as well as plant yield and growth (Blanco and Folegatti, 2005). Accurate direct measurements of plant canopy structure are laborious and numerous methods have been developed. These methods depend on, (1) morphological features of foliage elements to be measured; (2) accuracy required; (3) amount of vegetative material to be sampled; or (4) amount of time available, while using equipment. These methods can be divided into the following categories; (1) methods for leaf tracing, (2) methods based on matching standard leaf shapes and sizes, (3) methods for calculation based on linear measurements, (4) methods based on leaf area to mass relation and (5) methods of optical planimetric (Daughtry, 1990).

When discussing planimetric methods that use computers to measure leaves, automatic and semiautomatic techniques have been proposed. O'Neal et al. (2002) reported a method for measuring leaf area and defoliation by means of digital image analysis using a common scanner and public domain software. Li Zhichen et al. (2008) calculated leaf area with a non-destructive method through an algorithm in Matlab 6.0, utilizing a box with a hole on the top, where a camera was placed, a piece of white paper with a rectangle was then, placed on the flatbed where the leaf was covered in order to obtain high-contrast images. Igathinathane et al. (2006) developed a method using a computer to measure leaf area and perimeter, with software written using visual basic. They drew the leaf outlines of each specimen on the computer screen with a mouse and a keyboard. Another tool available is LAMINA, developed by Bylesjö et al. (2008), which works with images of scanned leaves; its output consists of the leaf area and shape parameters. However, the method was described only in general terms and it can not be reproduced with the information written in their published paper. Rico et al. (2009) calculated leaf area using digital photographs processed in Matlab and computer aided design (CAD) software but the calibration in this process is done by modifying code in the program and the segmentation is done manually. Even though the standard method to determine area, length and width of

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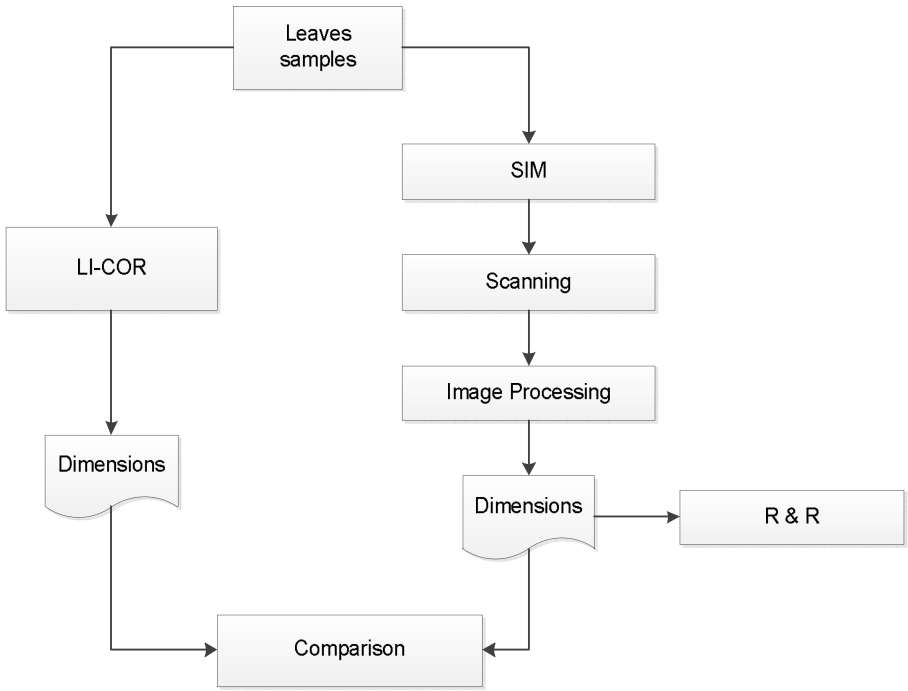

Figure 1. General methodology to test the proposed method.

leaves for agronomical applications is LI-COR 3000C leaf area meter (LI-COR Inc., Lincoln, NE); however, information regarding the shape and its evolution cannot be recorded by using this equipment. This information is fundamental to the study of the evolution of harvests. In the case of determining area using any kind of methodology, the results are referred to as total area, but due to the morphology of the surface the area should be referred to as apparent area.

The objective of this project is to propose an automatic method that is accurate and easy to implement based on image processing which could be used in physiological and agronomic studies, such as in Armstrong et al. (2006), Ciganda et al. (2009), Boussadia et al. (2010), Kahmen et al. (2008), Rossi and Pattori (2009) and Vohland et al. (2010). These require measurements of leaf areas, lengths and widths that can be compared with data obtained by means of standard methods and by establishing an algorithm with repeatable results. In the proposed method, the images are segmented using Otsu methodology and later, the noise is cleaned using a median filter. The resulting binary image is used for the calculation of the leaf area. A ratio between the white pixels counted on a leaf and the dots per inch (DPI) is established. The pixels of the leaf area are counted directly on the binary image. Before the length and width are obtained, the binary image is rotated to align the major axis of the leaf parallel to the y-axis. Finally, the maximum vertical and horizontal lines are obtained through directional erosions using a vertical or horizontal line, respectively, as the structuring element (Soille et al., 1996; Soille, 2003).

MATERIALS AND METHODS

The methodology followed for comparison of SIM was a standard method, in order to verify its measurement error as is shown in Figure 1. Different samples of leaves were cut and cleaned. Each leaf was scanned and its color bitmap file was acquired with the freeware InfarView 4.23. The images were processed with a personal computer with Intel® CPU 575 (2.0GHz) and software developed using Builder C++ 2009.

For SIM and LI-COR measurement comparisons a set of forty seven leaves was used, each leaf was measured three times using the LI-COR 3000C and LI-COR 3050C (LI-COR Inc., Lincoln, NE). LI-COR was used as a reference. The dimensions in points were obtained through the image processing methodology.

For the SIM reproducibility and repeatability study and in order to check the resolution of the proposed method, sixteen leaves of different species and thirteen leaves of *Citrus aurantifolia* of varying sizes were used, respectively. Each leaf was scanned twice with the HPColor LaserJet CM1312 and twice with the HP PSC 1210 with 200 DPI. Then, for each leaf, four replicates were obtained. The sample leaves of *Citrus aurantifolia* are shown in Figure 2.

LI-COR method

This equipment uses an electronic method of rectangular approximation for leaf area measurement. It is equipped with a scanning head that consists of a row of 128 narrow, red, light-emitting diodes (LEDs) spaced on 1 mm centers which creates resolution problems in the final image to be process due to scattering. These LEDs are sequentially pulsed, only one LED is lit at a time, in order to examine a particular grid cell in the row of the specimen, checking the adjacent rows one by one. This process can be performed

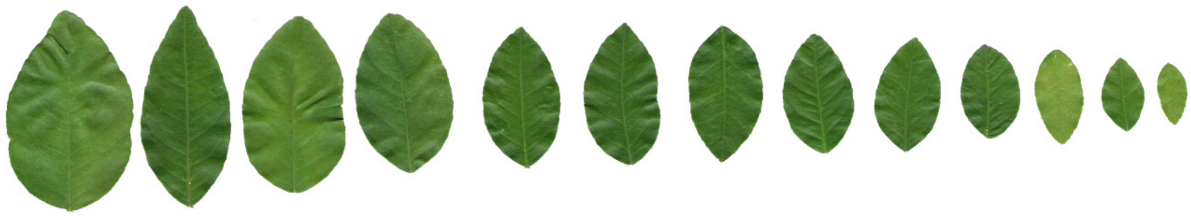

Figure 2. Sample leaves of *Citrus aurantifolia*.

manually by pulling a length of encoding cord by hand at a constant speed or automatically, with a specially included machine. If the sweeping speed is not kept constant, this may lead to measurement errors.

SIM description

The measurements of the scanned images were determined according to 200 DPI. This quantity was used to establish a ratio with the number of pixels for each area, length and width in the proposed method. If different resolution is used in the scanned images, this quantity needs to be specified in order to adjust the ratio. For the image processing method, each image was converted to grey tones and binarized with Otsu methodology. Then, a median filter was applied to clean the noise. Starting from the binarized and cleaned image, the area was obtained using a ratio with the DPI and the quantity of white pixels. Next, using the binarized image, that could be rotated to orient the major axis of the leaf parallel to the y-axis, the centroid was calculated and from it, the horizontal and vertical lines that cross it. Beginning with the length of each one of these lines, the maxima horizontal and vertical lines were determined. For this final measurement, horizontal and vertical lines were used as structural elements in directional erosions to ascertain the length and maximum width of each leaf. Figure 3 shows a flow diagram of the image processing of the SIM for each measurement parameter and Figure 4 shows a sample of the images of the leaves used for the comparison of the results of SIM and LI-COR.

Grey scale images

The scanned images were stored in the typical red green blue (RGB) color space (Gonzalez and Woods, 2008). However, for the segmentation process, it was necessary to work with grey scaled images. In order to perform this conversion, only the green band was considered because this band reveals more information about the leaf contours.

Segmentation

Otsu methodology was applied to perform an intensity transformation to reach an adequate value to binarize the image, where the total number of pixels in the image is N. In this case, these are assigned to two groups or classes using the pixel values. This method maximizes the variance between-classes. It establishes a threshold $T(k)=k$, with $0<k<L-1$ and two classes $c1$ and $c2$ as intensity values in the range of $(0,k)$ and $(k+1,L-1)$, respectively. L refers to the distinct intensity levels. The occurrence probability of the grey level i in the image is given by $p_i=f_i/N$; the zero order ω_k accumulated moment of the class c_k according to equation (1) and the first order μ_k accumulated moment of the class c_k as defined by equation (2). It also defines the variance between two classes σ_B^2 in a threshold as seen in equation (4) (Gonzalez and Woods, 2008) where μ_T is established in equation (3). The optimal threshold t^* is chosen when $t^* = Max\{\sigma_B^2(t)\}, 1 \le t \le L$ (Otsu, 1979; Gonzalez and Woods, 2008).

$$\omega_k = \sum_{i \in C_k} p_i \tag{1}$$

$$\mu_k = \sum_{i \in C_k} i p_i \,. \tag{2}$$

$$\mu_T = \omega_1 \mu_1 + \omega_2 \mu_2 \,, \tag{3}$$

$$\sigma_B^2 = \omega_1(\mu_1^2 - \mu_T^2) + \omega_2(\mu_2^2 - \mu_T^2) \tag{4}$$

In Figure 5, leaf samples are shown after applying Otsu methodology process. It is clear that some noise is still evident after the segmentation process as seen in the largest leaf image; therefore, an additional filtering process must be carried out.

Filtering

The binarized image may contain noise, such as little particles resulting from the previous process that need to be cleaned. For this purpose, a median filter was used; it replaces the value of each pixel in the image by means of the median of the intensity levels in the neighborhood (Gonzalez and Woods, 2008).

Area

The leaf area is calculated in centimeters using the DPI of the images. After the filtering process, the white pixels of the image are counted as (A) and a ratio with DPI is calculated in order to obtain the leaf area $(Area)$ as can be seen in equation (5).

$$Area = A(2.54^2 / DPI^2) \tag{5}$$

Leaf width and length

Once the image has been binarized and cleaned, the process to obtain leaf width and length is computed. The first part of this process is related to the alignment of the leaf, which includes points 1 and 2. If the alignment process is omitted the process to evaluate

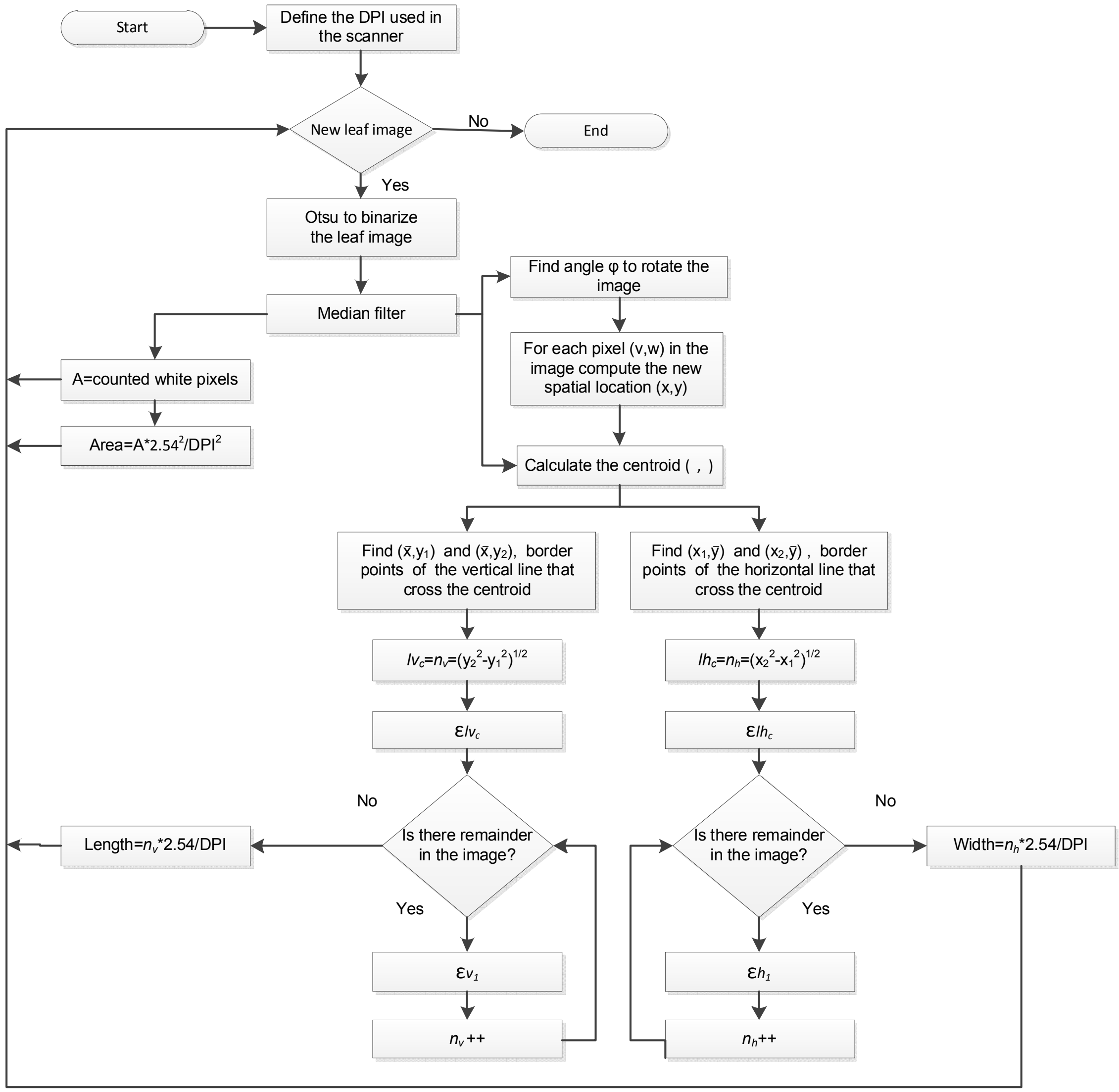

Figure 3. Flow diagram of SIM's image processing.

Figure 4. Sample leaves.

Figure 5. Sample leaves after binarizing.

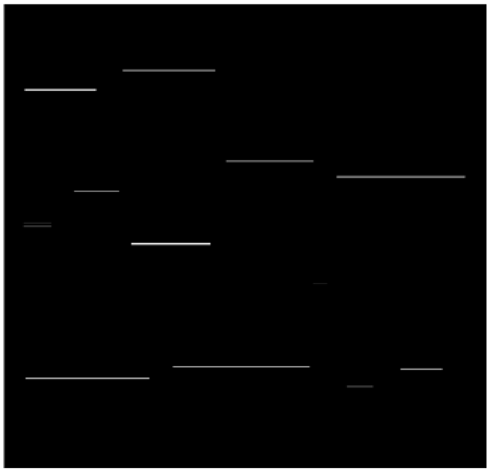

Figure 6. Sample leaves' width lines.

Figure 7. Sample leaves' length lines.

leaf width and length should begin at point 3. These steps are described as follows:

1. An angle φ to rotate the leaf image is obtained using second order central moments as in equation (6). The angle is calculated using the equations from (7) to (14), where $f(x, y)$ represents the grey level in the point (x, y), the point $(\bar{x}, \bar{y})$ is the centroid of the leaf image, $U(2,0)$, $U(0,2)$ and $U(1,1)$ second order centralmoments, $M(1,0)$ and $M(0,1)$ first order moments and $M(0,0)$ the zero order moment.

$$\varphi = \frac{1}{2}\tan^{-1}(2U(1,1)/(U(2,0) - U(0,2))) \quad (6)$$

$$U(1,1) = \sum_x \sum_y (x-\bar{x})(y-\bar{y}) f(x, y) \quad (7)$$

$$U(2,0) = \sum_x \sum_y (x-\bar{x})^2 f(x, y) \quad (8)$$

$$U(0,2) = \sum_x \sum_y (y-\bar{y})^2 f(x, y) \quad (9)$$

$$M(0,0) = \sum_x \sum_y f(x, y) \quad (10)$$

$$M(1,0) = \sum_x \sum_y x f(x, y) \quad (11)$$

$$M(0,1) = \sum_x \sum_y y f(x, y) \quad (12)$$

$$\bar{x} = M(1,0)/M(0,0) \quad (13)$$

$$\bar{y} = M(0,1)/M(0,0) \quad (14)$$

2. After obtaining φ, it is computed for each pixel (v, w) in the binarized image, the new spatial location (x, y) in the aligned leaf image through equations (15) and (16).

$$x = v\cos\varphi - w\sin\varphi \quad (15)$$

$$y = v\cos\varphi + w\sin\varphi \quad (16)$$

3. The new centroid $(\bar{x}, \bar{y})$ or center of mass in the alignment leaf image, which is the geometric center of the object' shape, is calculated again using equations (13) and (14).
4. The size of the longest horizontal erosion is n_h. This defines the quantity of points of the leaf width obtained through successive directional erosions of a line with a slope of 0° beginning with a structuring element of size lh_c; this size was obtained from the horizontal white line that crosses the centroid, followed by unitary erosions (ε_{h1}) until the data remaining in the image was found, that is, the final white points.5. The leaf width measured in centimeters is set as the ratio of n_h and *DPI* as in equation (17).

$$Width = n_h(2.54/DPI) \quad (17)$$

6. In a similar manner to the horizontal line, the size of the longest vertical erosion (n_v) is calculated. This variable defines the number of points of the leaf length. This is obtained through successive directional erosions of a line with a slope of 90°, beginning with a structuring element of size lv_c which also takes into consideration the size obtained from the vertical white line that crosses the centroid, followed by unitary erosions (ε_{v1}), until the remainder of the image is found.
7. The leaf length is also measured in centimeters and is set as the ratio of n_v and *DPI* as in equation (18).

$$Length = n_v(2.54/DPI) \quad (18)$$

Figure 6 and 7 show the images that resulted from the dilations of size n_h and n_v, respectively. The remainder in each case is used to rebuild leaf width or length. If there is more than one resulting line on one leaf, all of them have the same size.

Table 1. SIM versus LI-COR ANOVA comparison p-values summary.

Test for means	Area	Length	Width
ANOVA SIM vs LI-COR	0.990	0.962	0.983

Table 2. Linearity and bias tests p-values summary.

Concept	Area	Length	Width
Range measured	2.7-100.3 *cm²*	3.0-19.4 *cm*	1.0-9.3 *cm*
Bias test lowest p-value $H_0 : Bias = 0$	0.377 at 4.8 *cm²*	0.264 at 6.4 *cm*	0.248 at 4.0 *cm*
Avg. bias test p-value $H_0 : A.Bias = 0$	0.146	0.288	0.148
Linearity test p-value $H_0 : Slope = 0$	0.319	0.279	0.153

Table 3. R&R ANOVA Tables for *C. aurantifolia* leaves not aligned and aligned.

	Source	Not aligned					Aligned				
		df	SS	MS	F	p	df	SS	MS	F	p
	Leaves	12	3452.93	287.7442	3334.27	<0.001	12	3452.79	287.733	3339.05	<0.001
	Reproducibility	1	0.090	0.090	1.030	0.330	1	0.090	0.088	1.020	0.332
A	Interaction	12	1.040	0.087	0.860	0.597	12	1.030	0.086	0.860	0.598
	Repeatability	26	2.620	0.101			26	2.620	0.101		
	Total	51	3456.68				51	3456.53			
	Leaves	12	159.924	13.327	1132.43	<0.001	12	159.903	13.3253	1454.9	<0.001
	Reproducibility	1	0.014	0.014	1.220	0.291	1	0.002	0.002	0.270	0.616
L	Interaction	12	0.141	0.012	0.580	0.835	12	0.110	0.009	1.030	0.449
	Repeatability	26	0.524	0.020			26	0.230	0.009		
	Total	51	160.603				51	160.246			
	Leaves	12	55.3773	4.61477	9557.61	<0.001	12	55.4314	4.619283	8550.77	<0.001
	Reproducibility	1	0.002	0.002	5.040	0.044	1	0.003	0.003	5.170	0.042
W	Interaction	12	0.006	0.000	0.780	0.667	12	0.007	0.001	0.830	0.621
	Repeatability	26	0.016	0.001			26	0.017	0.001		
	Total	51	55.4016				51	55.4576			

Statistical analysis

1. The set of forty seven leaves of different species was measured with SIM and LI-COR and compared as two treatments with one way ANOVA. The hypothesis tested was $H_0 : \mu_{LI-COR} - \mu_{SIM} = 0$ and there was no evidence to reject the null hypothesis, being the lowest *p-value=0.962*. The results are shown in Table 1. These results suggest the treatments, in this case SIM and LI-COR measurement methods as general procedures were statistically similar.

2. Linearity and bias tests in the ranges of samples measured were prepared and the results are reported in Table 2. These tests were calculated using LI-COR measurements as reference. Linearity was evaluated based on bias along the range measured. A regression equation for the average bias at each reference was calculated and its slope was tested for $H_0 : Slope = 0$.

As shown in Table 2, linearity results were good for area, length and width. A t-test was used to verify $H_0 : Bias = 0$ using the pooled sample standard deviation method across ranges of the leaves measured. For this test, the lowest significance was p = 0.377, p = 0.264 and p = 0.248 for area, length and width, respectively. Therefore, area, width and length were found with no bias in the range. For the test of average bias $H_0 : A.Bias = 0$ the lowest p = 0.146 shows there was no bias in the average either. SIM was found with no significant bias and with no trends along the ranges measured.

3. The third statistical procedure was for repeatability and reproducibility assessment of SIM's results. In the context of this research, the reproducibility was mainly related with the variability caused by the position of leaves on the scanner's flat bed to capture the image. Repeatability assessed the variation induced by the imaging processing algorithm. Two experiments were designed and executed to verify the reproducibility and repeatability of the method. One used thirteen *C. aurantifolia* leaves and other included

Table 4. R&R variation breakdown for *C. aurantifolia* leaves not aligned and aligned.

Source of variation	Not aligned			Aligned		
	Area	Length	Width	Area	Length	Width
SIM R&R (%)	0.13	0.52	0.06	0.13	0.27	0.06
Repeatability	0.13	0.52	0.05	0.13	0.27	0.05
Reproducibility	< 0.01	< 0.01	0.01	< 0.01	<0.01	0.01
Leaf to leaf (%)	99.87	99.48	99.94	99.87	99.73	99.94
Resolution (categories)	38	19	59	38	27	57

Table 5. R&R ANOVA tables for different species leaves not aligned and aligned.

		Not aligned					Aligned				
	Source	df	SS	MS	F	p	df	SS	MS	F	p
A	Leaves	15	49714.4	3314.29	80662.9	<0.001	15	49714.5	3314.3	70486.5	<0.001
	Reproducibility	1	0.500	0.490	12.000	0.003	1	0.400	0.370	7.800	0.014
	Interaction	15	0.600	0.040	25.100	<0.001	15	0.700	0.050	16.900	<0.001
	Repeatability	32	0.100	0.000			32	0.100	0.000		
	Total	63	49715.5				63	49715.7			
L	Leaves	15	1210.74	80.7158	4468.91	<0.001	15	1310.46	87.3637	11110.09	<0.001
	Reproducibility	1	0.150	0.146	8.100	0.012	1	0.040	0.039	4.900	0.042
	Interaction	15	0.270	0.018	0.940	0.538	15	0.120	0.079	7.100	<0.001
	Repeatability	32	0.620	0.019			32	0.040	0.001		
	Total	63	1211.77				63	1310.65			
W	Leaves	15	484.583	32.3056	152122	<0.001	15	480.895	32.0597	239722	<0.001
	Reproducibility	1	0.001	0.001	5.000	0.046	1	0.000	0.001	4.000	0.074
	Interaction	15	0.003	0.000	1.000	0.851	15	0.002	0.000	4.000	0.001
	Repeatability	32	0.011	0.000			32	0.001	0.000		
	Total	63	484.599				63	480.899			

Table 6. R&R variation breakdown for different species leaves not aligned and aligned.

Source of variation	Not aligned			Aligned		
	Area	Length	Width	Area	Length	Width
SIM R&R (%)	< 0.01	0.11	< 0.01	< 0.01	0.03	< 0.01
Repeatability	< 0.01	0.09	< 0.01	< 0.01	0.01	< 0.01
Reproducibility	< 0.01	0.02	< 0.01	< 0.01	0.02	< 0.01
Leaf to leaf (%)	99.99	99.89	99.99	99.99	99.97	99.99
Resolution (categories)	215	41	220	217	89	407

sixteen leaves of different species. The experiments were done with two different scanners and each leaf was measured twice with each scanner. Images obtained were processed with and without alignment. Repeatability and reproducibility were evaluated using theanalysis of variance method. Results for *C. aurantifolia* leaves are shown in Tables 3 and 4. The results for the different species leaves are shown in Tables 5 and 6. For *C. aurantifolia* same species leaves results were similar for images aligned and not aligned except for length. This is explained due to the fact that, the length axis is the reference for the alignment process. As this was the lowest resolution measure its categories increased from 19 to 27 when images were aligned. SIM error was higher for length and also it reduced from 0.52 to 0.27% when alignment was applied to the images. Statistical significance of SIM was only important for width $p = 0.04$ and it had a slight decrease of the resolution when images were aligned from 59 to 57. Again, this is explained due to the fact that, the alignment axis was length; therefore, images aligned move the reference for width measurement adding some variation to this result. For different species leaves there were a consistent improvement in all indicators when images where aligned before they were measured. Lowest p-value for SIM significance increased from $p = 0.003$ to $p = 0.014$. This means the reproducibility error induced by the leave's position on the scanner reduced when alignment was implemented. Error as a proportion of variation also decreased from 0.11 to 0.03% and resolution increased from 41 to 89 for the length. For both sets, same species leaves and different species, the area resolution remained almost equal as alignment only affects length and width results. Total error caused by SIM as a complete measurement method was ≤0.52% when images were not aligned. This result suggests, it had very low error level for the samples and ranges measured. Categories were ≥19 also supporting the robustness of SIM even with no alignment.

RESULTS AND DISCUSSION

In this investigation by using the proposed method of image processing that the area, length and width of leaves can be obtained with results comparable to those obtained with a standard method. This study has merit most especially for studies where funding for specialized equipment and software is limited. The results were compared with those achieved by the use of LI-COR, which is standard equipment. Furthermore, a linearity test was done showing that there was no bias between these. Based on the results of the R&R tests, it was noted that, when the leaves were aligned the precision of SIM, became more accurate even when the leaves are aligned manually by the user and the step of aligning is omitted in the image processing method, the results were still acceptable. The measurement of same species leaves was the severest R&R test for SIM and in it the variation due to the measurement system was $R\&R_{SIM} = (0.27, 0.52)\%$ with the highest result for not aligned samples. In the same assessment $Resolution_{SIM} = (19, 27)\ Categories$, here the lowest was for not aligned samples. This suggests that, SIM could be an alternative way to measure leave area, length and width, requiring only standard office equipment and the image processing method proposed herein. Further evaluation may be desirable for samples of sizes under and above the ranges already in this research.

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