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Image Analysis-based System for Estimating Cotton Leaf Area

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMA made data analysis, managed the literature review and wrote the first draft of the manuscript. Author MFZ determined cotton leaves area by ImageJ software. Author MM participated in data analysis and participated in writing the first draft of the manuscript. Author HAE gathered the cotton leaves and calculated cotton leaves area by the graphical method. Author HHHT reviewed the measurements, participated in data analysis and participated in writing the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Leaf area is important for estimating biomass productivity, adaptation to the environment, nutrition, and soil-water relations. It also plays an important role in determining the proper application rates of insecticides and fungicides. Image processing is considered one of the best methods for estimating the leaf area of a plant as it is inexpensive and saves time. In the image processing method, leaf area is calculated through pixel number statistics by counting the number of pixels in the leaf area region of digital images. In this study, a simple system based on image analysis using the ImageJ software application was developed to estimate cotton leaf area. Two hundred and forty Egyptian cotton (Giza 86) leaves were captured using a digital camera. These leaves were collected randomly from different heights and different fields at Kafer El-Dawar center, El-Behera

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Governorate, Egypt. The results obtained using the proposed method was then compared with those obtained using the graphical method. There were only minor differences between both sets of results. The proposed method is feasible and practicable for estimating cotton leaf area as it has only a small overall average absolute relative error of 3.46% compared to the graphical method. Further, the proposed method is rapid, simple, and inexpensive.

Keywords: Graphical method; digital camera; ImageJ.

1. INTRODUCTION

Leaf area is considered an important indicator in the analysis and assessment of the growth and estimation of the production potential of a plant. Leaf area is also important in estimating biomass productivity, adaptation to the environment, nutrition, and soil-water relations [1]. In addition, it plays an important role in determining proper application rates of insecticides and fungicides [2]. Furthermore, leaf area knowledge can be useful in estimating the amount of chemicals to be sprayed for disease and pest control [3]. Thus, a simple, rapid, accurate, and non-destructive method for the estimation of leaf area may be useful for determining the relationship between leaf area and plant growth rate [4,5].

Egypt produces cotton in a wide quality range and staple lengths from 29 to 38 mm. Cotton cultivars in Egypt are either extra-long staple (i.e., Giza 45, Giza 87, and Giza 88) or long staple (i.e., Giza 86, Giza 80, and Giza 90) [6]. Moreover, Egyptian cotton is of a peculiar type that is characterized by high quality, and has gained a worldwide reputation for more than a century and half as being of the highest lint quality among world cottons [7]. The most important photosynthesis organ of the cotton plant is its leaves [8]. In addition, several factors such as leaf shape, size, area, and number of leaves per plant affect cotton seed yield [9].

Measurement of leaf area in crops such as cotton that have various types of leaf area is difficult, labour-intensive, and expensive because of the variation in number, size, and shape of the leaves [10]. Further, measuring instruments are very expensive and are often not available in developing countries [11,12]. Alternative indirect methods such as image analysis could be used to measure leaf area as they play an important role in the measurement of leaf dimensions [13] and in the detection of cotton pests and diseases [14]. In image analysis, digital cameras are used to acquire images that are then analyzed with appropriate software, either commercial or freeware. Digital scanners and cameras in combination with digital image processing software have replaced older leaf area measuring techniques [15].

Li et al. [16] showed that measuring leaf area based on image processing is more accurate than estimating leaf area using the grid paper method. They developed an inexpensive measurement system using a camera and a personal computer. The developed system consists of a wooden box with a camera in a hole in the center of the top face. The distance between the lens of the camera and the bottom of the box was 45 cm. The camera was set vertical to the flat surface and the lighting conditions were controlled using natural light focused on the leaf under a camera to take photos.

Patil and Bodhe [17] developed an area measurement method for Betel leaf based on image processing. They comparing the results with those of the graphical area measurement technique and showed that the method is accurate, with only a small relative error.

Ahmad et al. [15] evaluated various methods for measuring leaf area of selected winter cereals, specifically, wheat, barley, and oats. They compared digital image analyzer techniques that use Easy Leaf Area and ImageJ software with the manual method. The results showed a difference between the values recorded by the digital analyzer techniques and the manual method. The coefficient of determination (R^2) for the leaf area measuring techniques manual versus Easy Leaf Area, manual versus ImageJ, and Easy Leaf Area versus ImageJ were in the range 0.53 to 0.72, 0.90 to 0.93, and 0.66 to 0.73, respectively, for different sowing dates for the selected winter cereals.

The objective of this study was to develop a rapid and simple computer vision-based method for measuring cotton leaf area. In the developed method, images of cotton leaves collected using an off-the-shelf digital camera was analyzed using ImageJ. The results were compared with measurements obtained using the graphical method.

2. MATERIALS AND METHODS

2.1 Collection of Cotton Leaves

The selected cotton plant was Giza 86. According to El-Feky and Hassan [18], the cotton type is long-staple. The leaves were randomly collected from four different cotton planting fields, located in Elnashw, Near Elkarakool, Elkhadra, and the Elkarakool regions, Kafer El-Dawar center in the Nile Delta in northern Egypt (latitude 31°7′52″ N, longitude 30°7′48″ E, elevation 6 m), El-Behera Governorate, Egypt. The neighbouring fields were cultivated with rice, maize, and cotton. Each cotton field was divided into 20 equal plots. An individual cotton plant was randomly selected from each plot. The height of each cotton plant was divided into three canopy layers, in accordance with Alarcona and Sassenrath [19]: Bottom layer from zero (ground level) up to 45 cm, Middle layer from 45 cm to 105 cm, and Top layer from 105 cm. One randomly chosen leaf was picked from each layer and marked using a pen marker. A total of 240 leaves were collected. The leaves were kept in plastic pages and reserved in an ice box to keep them fresh. Table 1 shows the leaf position on a cotton plant, image number, average plant height and field location. Each leaf was numbered according to its position on the plant and its location, as shown in Table 1.

2.2 The Image Acquisition System

The image acquisition system is depicted in Fig. 1. A hole was drilled in the center of the top face of the white plastic box (lighting room) and the camera placed in the hole. The diameter of the hole is slightly larger than that of the lens of the camera. The distance between the top and bottom face is 45 cm. The leaf was placed under the camera and illuminated by two 26 W fluorescent lamps as light source (lumen = 1250 +/-20%). All lamps (13 cm long) were situated above the leaf sample. No forming shadow was observed. A color digital camera, model Canon SX600HS, 18X Optical Zoom with 16 Mega Pixels was located vertically over the leaf sample. Samples images were taken against a white background, and manual mode with no zoom and no flash was used by the camera. Images were stored in JPEG format. The camera was connected to the USB port of a laptop provided with control software for image acquisition and acquired the digital images directly from the camera.

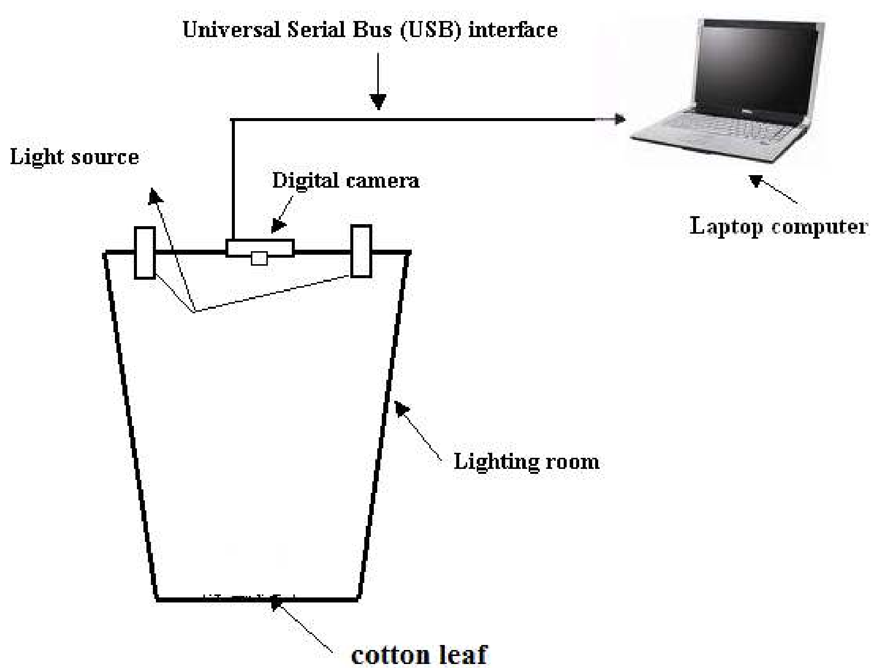

Fig. 1. Image acquisition system

2.3 Leaf Area Measurement Using ImageJ Software

ImageJ is a powerful program that was developed at the National Institutes of Health, USA. It is in the public domain, runs on a variety of operating systems, and is updated frequently. It can be downloaded for free [20]. The various buttons on the tool bar enable analysis of images. The window of the main menu of the ImageJ software is shown in Fig. 2. The numbered cotton leaf was placed against a white background and the image captured with the digital camera. The images were transferred to the laptop for further analysis through ImageJ.

2.4 Calibration of ImageJ

Various shapes (circle, rectangle, ellipse, etc.) with known area were drawn on a sheet of paper using a pencil. After capturing the shapes, they were analyzed using ImageJ. There was no difference between the results on record and those obtained via image analysis. The stored images were loaded into ImageJ and the area of the leaves determined. However, ImageJ was used to measure the area of only a selected region of each image via pixel count. The pixel count of the processed image depends on the distance between the camera and the object when the picture is taken [17]. The smaller the distance is, the larger the pixel count is. In this study, the fixed distance between the digital camera and the cotton leaf was 45 cm. A reference object is an object with a known area that is needed to translate the pixel count area. In this study, the metal Egyptian pound coin was chosen as the reference object.

Table 1. Leaf position on a cotton plant, image number, average plant height and field location

Leaf position on cotton plant	Image number		Average plant height (cm)	Field location
	From	To		
Top layer	1	20	140	Elnashw
Middle layer	21	40		
Bottom layer	41	60		
Top layer	61	80	155	Elkhadra
Middle layer	81	100		
Bottom layer	101	120		
Top layer	121	140	160	Near Elkarakool
Middle layer	141	160		
Bottom layer	161	180		
Top layer	181	200	165	Elkarakool
Middle layer	201	220		
Bottom layer	221	240		

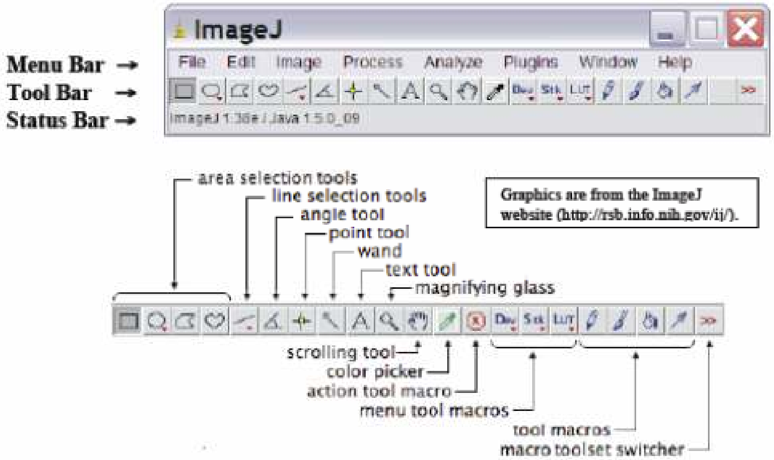

Fig. 2. Window of main menu of the ImageJ software

2.5 Leaf Area Calculation

First, the number of pixels in the leaf region and the known object region were calculated by selecting a threshold color from the image tab on the toolbar, and then the leaf image was generated in red. Then, the selection order was executed to select the green region, as shown in Fig. 3. By selecting the analyze tab on the toolbar, the leaf area result in pixels was presented, as shown in Fig. 4.

Fig. 3. Selecting the green region of the leaf

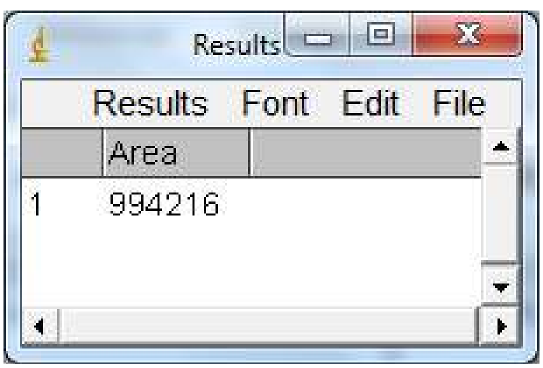

Fig. 4. Leaf area result in pixels from ImageJ

In this study, the metal Egyptian pound coin was chosen as the reference object. The actual area of the object is as follows:

$$A = \pi \times \left(\frac{d}{2}\right)^2 = 3.14 \times \left(\frac{2.5}{2}\right)^2 = 4.90625\, cm^2 \quad , \quad (1)$$

Where A is the area of the coin (cm^2) and d is the coin's diameter (2.5 cm). From its mage, the number of pixels making up the coin was 35120. Hence, one pixel = Area of coin/(total number of pixels) = 4.90625/35120 = 0.0001396996 cm^2. For the leaf sample, the total number of pixels was 994216. Hence, area of leaf = number of pixels × one pixel value = 994216 × 0.0001396996 = 138.89 cm^2.

The absolute relative error was utilized for evaluation of the image analysis method compared to the graphical method. However, the absolute relative error of a measurement signifies how large the absolute error is in relation to the correct value. More specifically, absolute relative error is the ratio of the absolute error of the measurement to the accepted measurement. In this study, absolute relative error percentage (E, %) was calculated using the following formula:

$$E = \frac{|A_G - A_P|}{A_G} \times 100 \quad , \quad (2)$$

Where A_P is leaf area, calculated by image analysis method and A_G is the leaf area calculated by graphical method.

3. RESULTS AND DISCUSSION

To evaluate the performance of the proposed measurement system, leaves were collected from different fields and different positions on cotton plants. The area of each leaf was calculated using a digital planimeter (graphical method, actual leaf area) and the standard measurement was obtained from previous work [21]. In the graphical method, the leaf to be measured was placed on graph paper. Then, it was outlined with a pencil accurately and carefully on the graph paper. The area was then measured using a digital planimeter, as shown in Fig. 5.

Table 2 lists the measurement results for the standard method (graphical method) and the proposed method with absolute relative error percentage for mean values. The absolute relative error can compare values and describe the accuracy of the measured values. The absolute relative errors of the leaf area were calculated using equation (2). When the absolute relative error value is small, the accuracy is high. However, relative error increases if the leaf has cuts due to insect bites, color patches due to diseases, or other stresses [17]. Moreover, the absolute relative error may vary because of leaf

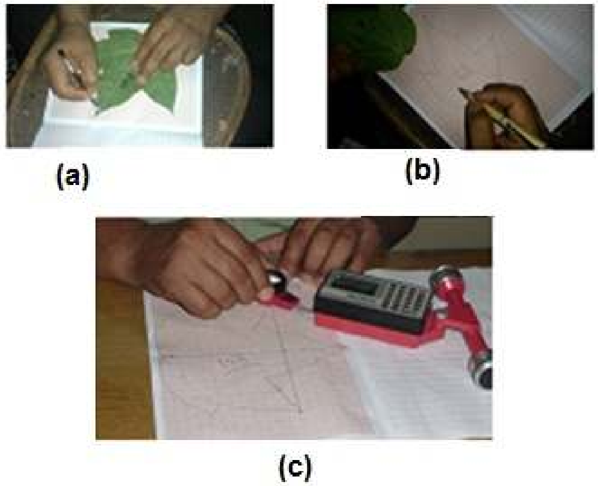

Fig. 5. Measuring the actual area using a digital planimeter (graphical method)
(a) Drawing an actual cotton leaf on a graph paper by a pencil
(b) The outline of an actual cotton leaf
(c) Measuring the actual area by passing a digital planimeter on the outline of a cotton leaf

deformation, incomplete image, and inaccurate image. Thus, some area will have to be removed from the leaf, resulting in a pixel count that is less than the true pixel count of the selected region; this gives an incorrect value for the computed area.

As can be seen in Table 2, there is a variation in the absolute relative error for leaves collected from different layers and locations. This may be attributed to the size of the leaves. With the implemented simple system the overall average relative error between area measurement by the proposed system and the graphical method was typically 3.46%, as shown in Table 2, which is very small.

Patil and Bodhe [17] compared the simple image analysis method with the graphical method for sugarcane leaf area and found that the simple image analysis method was accurate with a small relative error (2.9%). The results show that there is good agreement between the values recorded by the proposed system and that of the graphical method. The coefficient of determination (R^2) for leaf area measuring techniques for the graphical method versus the proposed system was 0.999, as shown in Fig. 6, using the average values for different leaves.

Table 2. Comparison of mean leaf area results, measured by the proposed system and the graphical method

Field location	Leaf position on cotton plant	Leaf area		Relative absolute error (%)
		Graphical method (actual leaf area)	Image analysis	
Elnashw	Top layer	77.22	73.79	4.45
	Middle layer	112.80	109.56	2.87
	Bottom layer	145.80	140.73	3.48
Elkhadra	Top layer	87.83	84.19	4.14
	Middle layer	180.82	176.88	2.18
	Bottom layer	193.34	188.96	2.27
Near Elkarakool	Top layer	84.93	80.34	5.40
	Middle layer	165.86	159.11	4.07
	Bottom layer	217.21	210.13	3.26
Elkarakool	Top layer	132.51	129.85	2.00
	Middle layer	184.89	179.67	2.82
	Bottom layer	210.75	201.00	4.62
Overall average relative error (%)				3.46

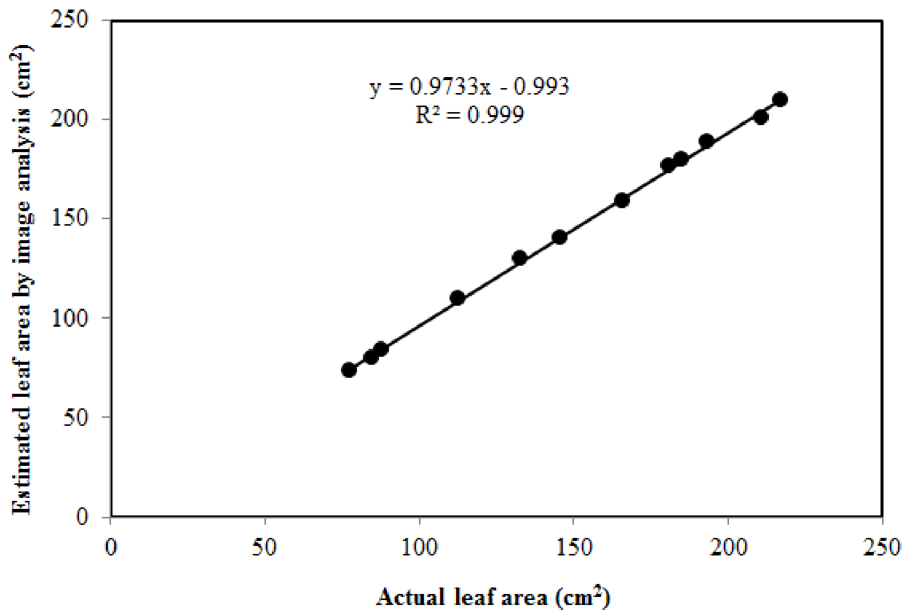

Fig. 6. Relationship between leaf areas measured by the graphical method versus the proposed system

4. CONCLUSION

Experiments were carried out using 240 cotton plant leaves. The coefficient of determination for leaf area measurement by the graphical method versus the image analysis-based system method was 0.999. As studies on plant growth and development continue to increase, the image analysis-based system method will be a very useful tool for estimating the leaf area for cotton plants without requiring additive expensive devices. It is inexpensive and can utilize any existing computer and digital camera; therefore, it does not require additional hardware. The proposed method will be valuable in achieving accurate area calculation and estimation of disease severity for the application of pesticide to cotton plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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