

Comparison of different Methods of In-situ Leaf Area Measurement of Betel Leaf (*Piper betle* L.)

Amar Kumar Dey, P. Guha, Manisha Sharma, M. R. Meshram

Abstract: The knowledge of leaf surface area is important for the evaluation of crop performance. Leaf area is a significant variable in many models describing the analysis of Crop growth, photosynthetic efficiency of the plant, plant nutrients and pest management, etc. Therefore, it becomes essential to study instruments and methods for measurement of leaf area which are quick, efficient, accurate, easy, convenient and cost-effective. This leads to two methods of leaf area determination of which the non-destructive methods are preferred to the destructive methods on many grounds. The present paper reviews and compares five ground-based techniques for in-situ leaf surface area measurement based on percentage error and execution time. The result shows that all ground based leaf surface area measurement method is reliable, but the method based on Digital Image Processing (DIP) approach is the most accurate, convenient and fast technique.

Index Terms: Leaf area, Digital image processing, Counting grid, Accuracy, Growth analysis.

I. INTRODUCTION

In modern agronomy, a diverse arenas of technology, such as process control, electronics, artificial intelligence, and biotechnology are applied to increase economic output [1]. The measurement of leaf area is of fundamental importance to agricultural and ecological research. The accurate measurement of leaf area can help us quickly understand, leaf development and leaf area growth rate, photosynthesis, and water relation of leaves, leaf to fruit ratio and to develop a reasonable way of cultivation, pruning, and fertilizer application, etc., programs [2]. Many methods of measuring leaf area have been studied, but most of them are a combination of several measurement models with complex and difficult mathematical equations. In the existing review, an attempt has been taken to classify all the basic leaf area measurement techniques in two categories as direct and indirect methods. A brief organizational overview is as follows. The methodology used is described in section II that includes five different methods of leaf area measurement which are as follows, Counting grid squares, Length width regression equation, Using planimeter instrument, Paper

weight method and Digital image processing based method. Results and discussion are highlighted in section III. The Conclusion is summed up in the last part.

II. METHODS

Betel leaves (*Piper betle* L.) have been taken as the experimental material in the present study, because it is a leafy crop. The leaves are directly sold in the market after plucking [1]. Now, since the leaves are the economic part of the plant therefore, determination of leaf surface area assumes significant importance. The existing leaf area measurement techniques and principles are categorized into two groups: Direct and Indirect leaf surface area measurement methods (Fig.1):

A. Direct methods

Direct methods are destructive approaches, which include the measurement of all the different leaf areas, are precise, but labor intensive. Therefore, have limited application and is suitable for short status ecosystem such as greenhouse system. But, is difficult to apply to the study of the large geographical area, over large leaf samples and for analyzing the successive growth cycle of a leaf. The two basic steps involved in direct measurement are, sample harvesting (leaf collection) and leaf area measurement [3]. Nevertheless, area measurement based upon direct approach is considered as the accurate measurement and therefore, considered as an important calibration method for indirect leaf area measurement.

B. Indirect methods

Indirect method, first involve in developing a relationship between few plant features with the actual leaf surface area. Then the significant plant features are estimated using other variable. They are generally rapid, amenable to automation, and thereby agree for a larger sample to be acquired and thus, becoming gradually more imperative [4] and preferable.

The indirect approaches discussed are non-destructive and play important role in the successive leaf area measurement particularly for leaf growth analysis. One hundred Betel leaf samples with the corresponding area measured using five different techniques is described in the following paragraphs:

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TABLE I. COMPARISON OF DIFFERENT METHODS OF DETERMINATION OF LEAF AREA

S. No.	Methods	Relative Percentage Error (cm ²)	Processing time (Seconds)/Leaf
1	Counting Grid Squares (CGS)*	0.00%	358.88
2	Length Width Regression Equation (LWRE)	8.94%	148.9
3	Using Planimeter Instrument (PI)	-5.47%	64.87
4	Paper weighing (PW)	-3.19%	30.61
5	Digital image processing based (DIP)	-1.26%	7.85

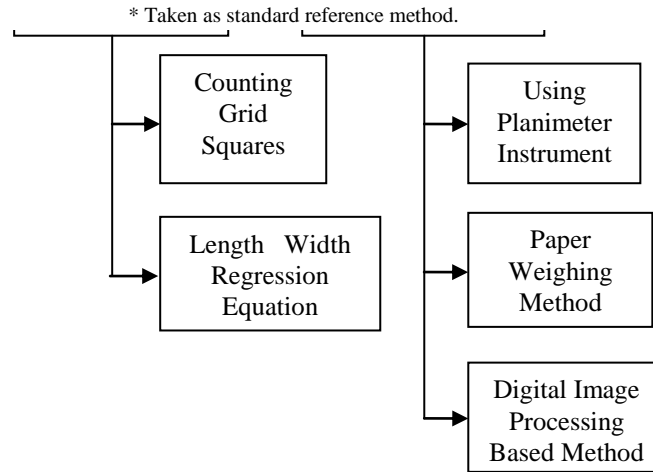


Fig. 1. Classification of Direct and Indirect methods for determination of leaf area

A: Counting Grid Squares (CGS)

One approach to find the leaf surface area is to sum the number of square cm. needed to cover a leaf surface. To count the number of square cm., a simple approach is to construct unit squares within the interested area. For example, the graph paper that has 1-centimeter square grid lines can be used for this purpose. The estimation of leaf surface area is obtained using following steps [5].

- (1) Place the leaf to be measured on graph sheet that has 1 cm² square grid lines. Outline the perimeter of the leaf on the graph paper.
- (2) Estimate the number of square blocks are enclosed inside the leaf outline.
- (3) Finally, the leaf surface area is calculated using equation (1), [6]:

$$Leaf\ Area = NGS \times OGA \dots\dots\dots (1)$$

Where, *NGS* is the number of grid squares inside the leaf outline and *OGA* is the area of single square grid. The precision of this technique is dependent on, how the areas covered by half grid square and quarter grid squares are accurately interpreted.

B: Length Width Regression Equation (LWRE)

Calculating leaf surface area from regression equations, involving simple measurements of leaf sizes is an economical, rapid, and a nondestructive option for correctly assessing the leaf size.

In order to develop an appropriate model for estimating leaf surface area by means of leaf axial length and maximum width, we go through the following steps [7, 8]:

- (1) A translucent paper was placed on a leaf and its trace was marked.
- (2) The length and the maximum breadth of this trace were measured using a ruler.

A regression equation was used for this purpose, according to the following formula [9, 10]:

$$Leaf\ Area = f(L', W_d) \dots\dots\dots (2)$$

Where, *L'* is leaf height, *W_d* is leaf width and *f* is a function that can either be linear or polynomial.

The results show that the leaf area has the best relationship with the maximum width and length of the leaves in a power function mode. However, though the obtained results are most precise and accurate but, the function needs to be redefined for each and every species of leaf.

C: Using the Planimeter Instrument (PI)

The Planimeter consists of few important units. Its first unit comprises of stationary optical scanning system and secondly a Microcomputer System (MS) to facilities embedded and controlling functions. The leaf under observation is placed over a conveyer belt arrangement driven by servomechanism and controlled by MS unit. On each successive axial leaf sample propagation by 0.1cm, the optical scanner measures the maximum breadth and maximum length of the leaf sample and store the result in area register.

The Plan meter leaf surface area measurements are performed in the following steps: [11]

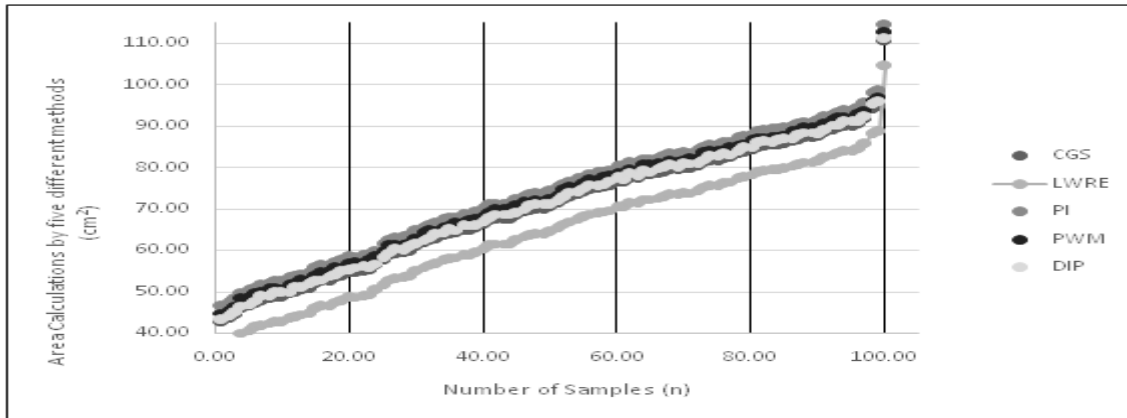


Fig. 2. A sample of hundred betel leaves used in the present work for evaluation of leaf area measurement using five different approaches.

1. To start the procedure place the leaf sample, over cover belt system (with the leaf tip just touching the optical scanner unit)
2. The MS unit successive update the present breath and length readings as the leaf cover an axial displacement of 0.1 cm and update the incremental length register.
3. A non-zero breath measurement, indicates the completion of scanning process. Finally, the area is estimated using equation 3,

$$\Delta a = \sqrt{4\Delta l^2 + (W_0 - W_1)^2} \dots\dots\dots(3)$$

Where, Δa is area increment, Δl^2 is length increment, W_0 is current breadth measurement and W_1 is the previous breadth measurement. The procedure is repeated (step 1 to step 3), for each leaf sample and lastly, the area result is displayed on the display device. An electronic planimeter thus facilitates easy and quick measurement of leaf surface area.

D: Paper weighing (PW) method

It is the simplest approach, based on sketching leaf shapes on a 0.5 cm² graph paper, and determining leaf surface area by measuring the weighing of the paper sheet with a highly precise electronic weighing machine [12].

In this method, the leaf sample is first detached from the mother plant and positioned on 0.5 cm² graph paper. After that the square cm. graph paper is cut-out along the traces of leaf perimeter outline. Then the mass of square cm. graph

and in the reference image. Finally, the leaf surface area is calculated using equation 5, [16, 17]:

$$L_a / L_p = B_a / B_p \dots\dots\dots(5)$$

Where, L_a is the actual leaf area, L_p is number of pixels in leaf image, B_a is reference background area and B_p is number of pixels in the background image.

F: Evaluation indexes

Relative Percentage Error (RPE), is used as one of the evaluation parameters to estimate the accuracy of five

paper is matched to the mass of known area on the identical paper. Finally, leaf surface area is calculated by making use of the following relationship [13, 14].

$$\text{Leaf Area} = PT/UW \dots\dots\dots(4)$$

Where, PT is the mass of the graph paper trace of the leaf and UW is a constant of the paper (mass of unit area). This method is also laborious and time-consuming when used on a large number of leaves [15].

E: Digital image processing (DIP) based method

The cognitive vision approach plays a vital role in the study and interpretation of optically detected information. DIP involves the statistical manipulation of digital information for refining the image qualities with the aid of a microcomputer.

DIP method was developed for measuring leaf areas with a high degree of precision and ease of operation, using an inexpensive digital camera coupled to an IBM-PC. The data analysis was performed using Matlab.

The step required for calculation of leaf area is described as below:

- (1) Acquire the image of the leaf.
- (2) Convert it to a gray image.
- (3) Segment the leaf image and reference image (i.e.15cm x 15cm background)
- (4) Compute the number of pixels in a sample leaf imag

different leaf surface area measurements. RPE compares the amount of error to the total amount of measurement and is given by equation (6):

$$\text{Relative Percentage Error (RPE)} = \frac{(\text{True measurement} - \text{Observed measurement})}{(\text{True measurement})} \times 100 \dots\dots(6)$$

The true measurement is the leaf area obtained from CGS method, Observed measurement is obtained from area measurement from LWRE, PI,



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PW and DIP method.

The processing time is the second important parameter used in the evaluation of leaf area measurement it is calculated using an electronic stopwatch, which is started at the beginning of area measurement process and stopped after the completion of the process.

III. RESULTS AND DISCUSSION

The result of five different methods of leaf surface area measurement of betel leaf are discussed below. Data sets of hundred fresh leaves of betel vine were randomly selected and used in all the subsequent measurements. Counting grid square method is considered as the reference method in relative error calculation. One hundred Betel leaf samples

with the corresponding area measured using five different techniques is shown in fig. 2.

Table I, shown below gives the relative percentage error (RPE) and processing time (PT) for each method. Fig. 3 provides a cluster bar chart to compare mean percentage error, of five different methods. A negative RPE indicates an over estimation of leaf surface area, as appeared for PI, PW and DIP method. Whereas, a positive RPE indicates an underestimation of leaf surface area, as appeared for LWRE (Table I).

Every method, though reliable, but the processing time involved in method 1 (CGS) and method 2 (LWRE) are the highest; method 2 (LWRE) involves a number of diverse equations and one equation is only appropriate for one specific variety of crops; also, the method 3 (PI) and the method 5 (DIP) are inexpensive and method 1-4 are destructive.

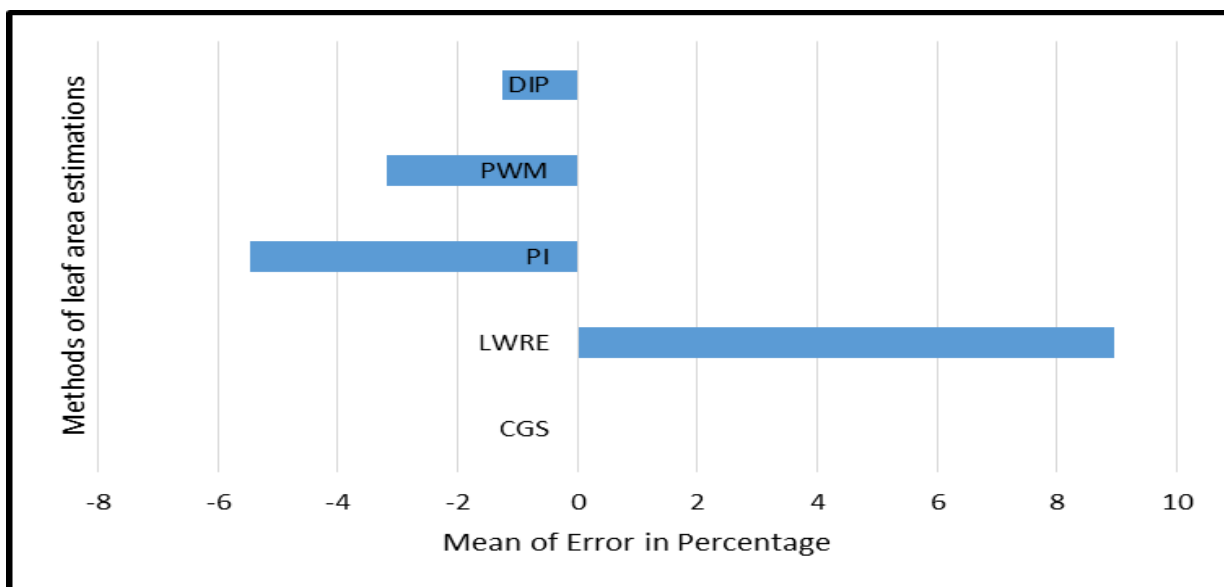


Fig. 3: Comparison of different methods on the basis of mean percentage error

The grid counting methods have very maximum accuracy, but need high processing time to measure leaf surface area. Therefore, if the crop leaf length and breadth is relatively high. Then, regression equation (LWRE) methods are simple and precise, but in order to estimate the total leaf surface area, one still has to measure both in axial and lateral direction for a single leaf sample, which is highly laborious. Planimeter with a graphic table are operator-interactive; they require mechanical sketching of the leaf sample. The Paper weighing method is an exhaustive work for the operator, which is also time consuming and the situation become even worse if the sample leaves that are highly damaged by insect, pest, and diseases. The PW is also subjected to error due to lack of exact drawing of leaf perimeter, lack of even cutting along the drawn outline, and lack of accuracy in weighing.

The precision and accuracy is very high in the Digital image processing (DIP) method of leaf surface area estimation as shown in Fig. 3. Also from fig.4, it can be concluded that DIP method is about 97.81% faster than CGS method. The DIP area measurement consumes less processing time as shown in Fig. 4. Therefore the leaf surface area estimating using Digital image processing (DIP) technique is

a cheaper, quick, and non-destructive alternative for accurately assessing Betel vine leaf surface area.

The Digital image processing technique is a compact and portable device for the accurate measurement of leaf surface area using a non-destructive approach. In DIP technique the sample image acquisition unit comprises of a high resolution digital camera and a white background board with a square shape reference piece of paper. The data thus acquired is send to the Laptop for image processing and saved for future use. For sample leaf image acquisition flat bed or laser scanner can also be used. The DIP techniques are very versatile and can be extensively used in the area of leaf surface area measurement and agronomy studies.

IV. CONCLUSION

Among the five different methods examined for determination of leaf surface area of betel vine, DIP method was found to be the best method being cheap, easy, and inexpensive. This method can be used for automatic detection of betel leaf area in large quantities for agronomical studies and forestry research without using expensive instruments and laborious procedure.

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