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Evaluation of Leaf Area Parameter of Pomegranate (*Punica granatum* L.) Germplasm by using ImageJ in Comparison with Manual Method

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Abstract

The size and shape of leaves can vary significantly between different genotypes within the same species and they implicitly influence plant growth and therefore productivity. The aim of this study was to compare leaf area of 10 genotypes of pomegranate (*Punica granatum* L.) through image segmentation procedure using ImageJ software. The data indicates large variation for leaf surface area characteristics from one genotype to another. During evaluation, leaf area observations of both the manual and ImageJ method were found positive and significantly correlated. The ImageJ based approach proved to be a reliable and non-destructive method for rapid estimation of leaf surface area, offering a valuable tool for research in physiological studies, irrespective of the genetic material under investigation.

Key words : Pomegranate, ImageJ, leaf area parameter, Correlation, Cultivars, non-destructive method.

Phenomics in present era plays an important role for studying large data sets. In any study the prime parameter for estimating photosynthesis is through leaf parameters. Among the different parameters leaf area is one of the most important parameter in estimating the sink source ratio and indirect estimation of stress tolerance. The leaf area will tell us the photo oxidative efficiency, water use efficiency, and drought avoidance. The use of semi-automatic tools will always help in estimating parameters at large scale in comparison to manual methods. Pomegranate is a dryland commercial fruit crop in arid and semiarid regions. Where many farmers livelihood is depends on this crop due its water and nutrient use efficiency (Saroj and Kumar, 2019). In any crop plants, the water use efficiency is indirectly related to its productivity. In pomegranate, leaf area parameters will help in estimating DUS characteristics like leaf size, shape, angle, and physiological parameters like water, nutrient use efficiency. Estimating large number of leaves for leaf area parameter through manual method is tedious and requires longer duration. Hence use of free software for estimating the leaf area will be of great use. Hence in this study, we compared the efficiency of ImageJ tool and standard graph paper method in estimating leaf area parameters.

ImageJ is an open access image analysis program used in the biological sciences and other allied sciences. ImageJ software helps in estimating the different parameters based on the photographs rather than manual observations. It doesn't involve the physical measurement (Rueden *et al*., 2017); it measures the data in the form of megapixels rather than standard units. All operations in ImageJ software tool are performed on the active image. It runs either as an online applet or downloadable application on computer with a Java 1.4. ImageJ equipped with an open architecture that allows better extensibility via java plugins. Custom acquisition, analysis and processing plugins can be

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developed using ImageJ written plugins to solve any image processing or analyse the problem. In this study, we assessed the leaf area of the samples using conventional standard graph method and ImageJ software. The values of both the methods were compared. The main objective of this study is to estimate the efficiency of ImageJ software in estimating the leaf area of pomegranate leaves.

Material and Methods

In the present study, plant material consists of 10 genotypes of pomegranate, includes both indigenous and exotic accessions. All the genotypes constituted the material in this study were maintained at the ICAR–National Research Centre on Pomegranate field Gene Bank. The lists of germplasm with features are detailed below in the Table 1. The five leaves of each genotype were collected and photographs of the leaves were stored for ImageJ analysis and observations were recorded by standard graphical leaf area estimation method. Later, the leaf area was estimated by ImageJ through the below protocol

The protocol of ImageJ analysis for leaf area parameter

1. Initially the photographs of each genotype leaf samples were stored.
2. Later photographs of the leaves were subjected to ImageJ software for leaf area estimation.
3. Before analysing the leaf area, the image processing is an important step, Image was converted into 8 bit and image color was adjusted by using the option color threshold.
4. Next, setting the scale i.e defining the measurements to the software. For example centimetre into megapixels, as the software accepts only megapixels data. It varies with the parameters.
5. During the image analysis, foremost step is background nullification.
6. Next, correction of background effects through colour threshold plugin.
7. Then the hue line has to be obtained by joining the ends of the leaves, through that it is able to cover whole leaf area.
8. Then area, mean and standard deviation of five samples were estimated by using the option measure.
9. And results were obtained in excel format.

Table 1. List of germplasm with their characteristic features

Genotype	Type	Fruit size	Fruit colour	Aril colour
1185	Wild	Small	YP	Pink
Khandhari	Exotic	Medium	YP	Light Pink
IC 318712	Wild	Small	YP	Pink
IC 318705	Wild	Small	YP	Pink
Bhagawa	Cultivated	Medium	Red	Red
Mridula	Cultivated	Medium	Deep red	Dark red
Alah	Cultivated	Small	Pinkish	Red
Maha	Cultivated	Small	Pinkish	Red
Patna 5	Cultivated	Medium	YP	Light pink
1180	Wild	Small	YP	Pink

YP = Yellow with pink

Results and Discussion

The mean values of all the genotypes were estimated for both the methods. The descriptive statistics were calculated for the sample data. The values are given in Table 2. Then the mean data was subjected to statistical analysis. The analysis of variance showed non-significant differences within population and significant differences between the groups of both ImageJ and graph method. The results are given below in Table 3 and the raw and processed leaf images are shown in Fig.1.

The individual genotype leaf area was

Table 2. The mean values of leaf area observed through graph and ImageJ software are given below

Genotypes	Graph method	ImageJ
Alah	4.341	4.328
Mridula	7.391	7.391
IC 318705	5.756	5.754
1180	9.895	9.903
Patna 5	6.226	6.227
1185	6.176	6.152
Maha	5.561	5.567
Bhagwa	5.830	5.804
Khan	8.059	8.049
IC 318712	5.55	5.55
Mean	6.721	6.716
Min	5.555	5.55
Max	9.895	9.903
SE	0.490	0.491
SD	1.470	1.474

Table 3. Analysis of variance of leaf area parameter between genotypes

Source of Variation	SS	df	MS	P-value
Between Groups	0.000202	1	0.000202	0.992912
Within Groups	44.86323	18	2.492402	
Total	44.86343	19		

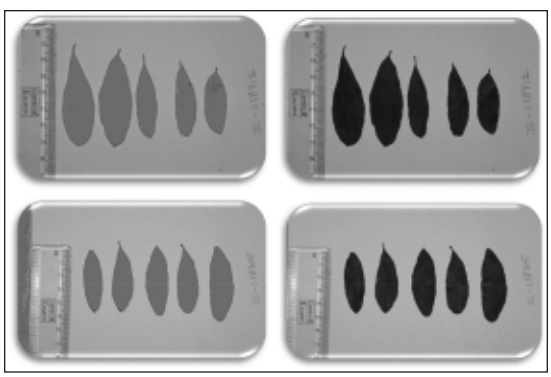

Fig. 1. The original image (right) vs processed images (left) through ImageJ software

measured through ImageJ and Graph based method was subjected to correlation analysis. The correlation analysis was found highly significant with positive correlation (Fig. 2 and Fig. 3). The regression analysis also exhibited perfect line fit for the software estimated values with graph estimated values. Based on the results, the ImageJ software can be used for estimating the leaf area parameter with 99 % accuracy which is on par with manual estimation. Later the correlation analysis was also done for individual genotypes for individual leaf samples values of both the methods. The results were found highly significant.

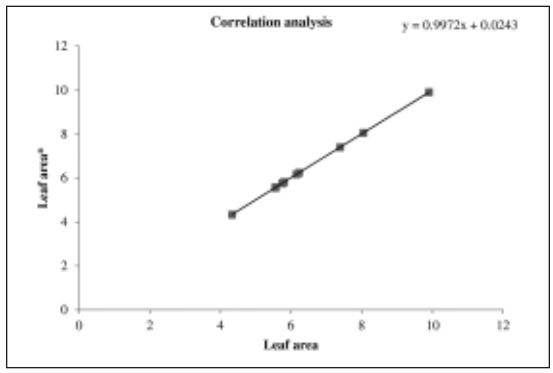

Fig. 2. The correlation analysis between the mean values of ImageJ and graphical method

The open access software ImageJ will ease the estimation of parameters like leaf size, shape and fruit parameters like fruit size shape which is tedious in nature as the sample is tiny size. (Agehara 2020; Alheeti et al., 2021). In this study, we tried to find the efficiency of ImageJ in comparison to graphical method estimation. The correlation analysis revealed a perfect correlation among two genotypes namely Patna 5 and IC 318712 indicates that leaf area estimation through ImageJ is perfectly correlated with manual method. This can be used as an alternative for the tedious manual method. The results revealed a perfect correlation between ImageJ and manual estimation (Starwarczky and Stawarczyk, 2015). Thus estimation of the values through ImageJ will reduce time and manpower involved

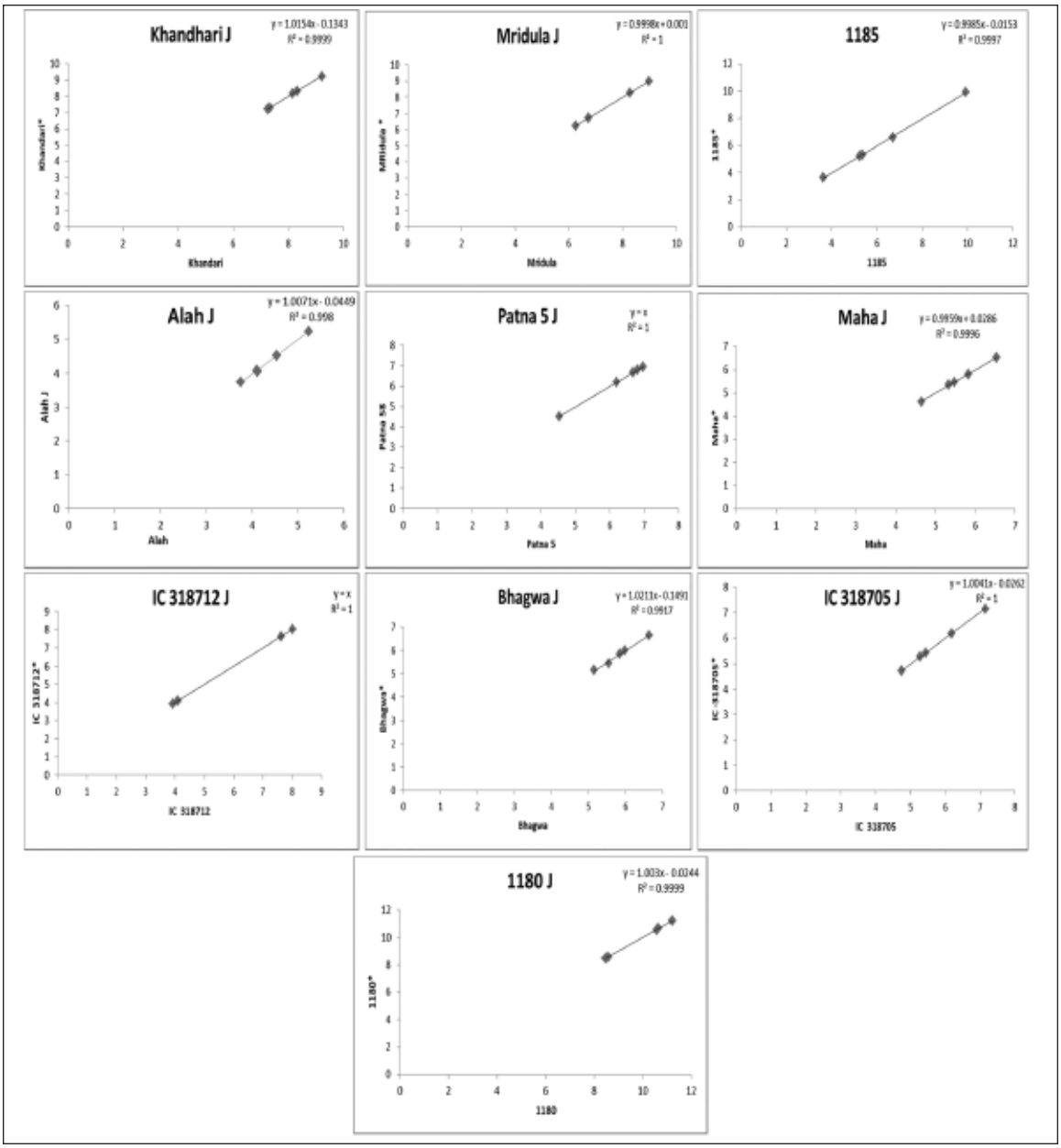

Fig. 3. The correlation analysis between estimated values of individual genotypes through ImageJ vs graphical method. *indicates values through ImageJ software

(Agehara 2020). Hence this software will be better alternative for leaf area estimation. The regression analysis also revealed clear fit between the ImageJ and manual estimation values; hence it can help in estimating the leaf area through photographs than measuring manually with better precision and accuracy (Sunoj *et al*., 2018).

In conclusion, the ImageJ with measure

plugin open software proves to be superior alternative for quick and efficient estimation of leaf area parameters with minimal human intervention. It significantly improves the accuracy and efficiency in measuring the these parameters, and its importance in agriculture and plant biology is paramount and cannot be underestimated.

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Conflicts of Interest

The authors declare no conflict of interest.

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