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An image analysis based system (Image J) for determination of leaf area in seven chrysanthemum varieties

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Abstract
Leaf area is an essential parameter in plant-based research as it affects various morphological and physiological aspects, including photosynthesis, partition assimilation, and biomass accumulation. Traditionally, graphical method and leaf area meter are used to determine leaf area, which is time-consuming and requires technical precision. A study was conducted in 2020 at IARI, New Delhi, for leaf area analysis using an image processing software, Image J®, on seven *Chrysanthemum morifolium* varieties. The procedure involves harvesting leaves of individual plants and placing them on a white sheet with a reference object. The photographs were captured using a digital camera and were processed using the software. White Star, a standard type variety had highest leaf area (48.03 cm^2) which was in sync with other parameters like stem length and diameter, and bud and flower diameter. This approach using Image J® software is a rapid, inexpensive, and reliable tool for determining leaf area with higher accuracy.

Keywords: Image J, leaf area, chrysanthemum, software, camera

1. Introduction

Leaf is considered an important organ for plant as it is the site for photosynthesis. Thus, leaf area is a crucial parameter for determination of plant nutrition, environmental adaptation, plant productivity and soil-water relations (Hajjdiab *et al*. 2010) [14] and is frequently used in plant based research experiments. Leaves are often associated with capturing light, gaseous exchange and thermal regulation (Fanourakis *et al.* 2017, Fauset *et al*. 2018, Zou *et al*. 2019) [15, 16, 18]. Furthermore, leaf area and size have been shown to influence the rate of plant growth (Robbins *et al*. 1987 and Montero *et al*. 2000) [19, 20]. Leaf area can be useful in estimation of amount of pesticides and fugicides to be sprayed according to the plant requirement (Suggs *et al*, 1960) [28]. Thus, a comprehensive knowledge on plant growth and development can be obtained through leaf area analysis (Padron *et al*., 2016) [21] which in turn can be utilized for in-field practices like fertilization and planting density (Toebe *et al*., 2019) [22]. In case of ornamental plants like chrysanthemum, variation in leaf shape and morphology can help in species identification and realization of aesthetic and decorative potential of the plant.

The conventional approaches for leaf area measurement are inclusive of the graphical method or using a leaf area meter. These procedures are usually time consuming and might introduce errors, especially when dealing with extremely small or narrow leaves, or leaves with serrations (Cornelissen, 2003) [23]. Using digitalized leaf images in appropriate software which can enable processing images and calculating leaf area can provide a reliable and viable option (Lucena *et al.* 2011) [24]. Digital imaging software's have been employed for various crops and have been documented in Coffee (Tavares-Júnior *et al*., 2002) [26], and Soybean (Adami *et al.* 2007) [27]. With regard to ornamental crops, Rose (Matloobi *et al.* 2019) [7] and Chrysanthemum (Fanourakis *et al*. 2021) [4] have been used as a model plant.

In recent years, various studies have been conducted in different crops using Image J as one of the analytical tool. In India, Patil and Bodhe (2011) [11] measured Betel leaf area using image processing Image J and concluded that method is statistically accurate with only a small relative error. Mollick *et al.* (2012) [8] quantified leaf parameters in 29 *Codiaeum variegatum* cultivars through Image J software. Analysis of variance suggested that significant variations were observed among the cultivars for all quantitative leaf parameters. Ramos *et al.* (2015) [13]

estimated areas of leaf blade of cotton, cashew, soybean and corn based on linear and dry weight measures, calibrated using ImageJ and the method was found appropriate for estimating area of damaged leaves. Padron *et al.* (2016) [21] developed polynomial models to estimate leaf area in *Capsicum annum*. The destructive method of leaf discs showed high correlation (r2=0.99) with the non-destructive method of ImageJ software. Dos santos (2016) [3] determined allometric equations in 1000 *Hymenaea courbaril* leaves for better estimation of leaf area using ImageJ as one of the destructive alternatives, and concluded that ImageJ software can replace the LI-3100 analyzer to determine the leaf area. Aboukarima *et al.* (2017) [1] analyzed 240 cotton leaves using ImageJ software and found only a small percentage of absolute relative error (3.46%) compared to graphical method. Cosmulescu *et al.* (2020) [2] compared length, width and surface area of 9 medlar genotypes using ImageJ, which provided a good nondestructive method for leaf surface area estimation. 100 *Anacardium humile* leaves were collected and scanned under ImageJ program, suggesting that leaf area can be determined by image analysis software and linear measurements are correlated to leaf area (Gomes *et al.*, 2020) [5]. Pachecho *et al.* (2020) [10] suggested that in Zucchini, nondestructive method (ImageJ) with caliberation is required for most accurate leaf area estimation. ImageJ was found statistically accurate with NSE (Modelling Efficiency Index) and PBIAS (Percentage of Bias). Martin *et al.* (2020) [6] compared two methods of estimating leaf area (leaf area meter LI-COR 3100 and ImageJ software) in oats. The results were highly correlated, and variances were homogeneous, thus concluding that ImageJ software can be used instead of the leaf area meter.

The goal of present study was to introduce new image processing software, ImageJ (http://imagej.nih.gov/ij/). This is open source software package that enables user to calculate automated leaf area using digital imaging by a simple digital camera. Chrysanthemum, a worldwide popular ornamental crop was used as a model plant as it has irregular shape and size with respect to leaf morphology (Gao *et al.*, 2020) [25]. Seven chrysanthemum varieties (Autumn Pink, Bronze Turner, Sparrow, Autumn Yellow, Diana Orange, White Star and Zembla) were studied using this software.

2. Materials and Methods

2.1 Materials

The study was conducted in Indian Agricultural Research Institute, Pusa Campus (New Delhi) in 2020-21. Seven *Chrysanthemum morifolium* L. varieties (Autumn Pink, Bronze Turner, Sparrow, Autumn Yellow, Diana Orange, White Star and Zembla) were encoded as C_1, C_2, C_3, C_4, C_5, C_6 and C_7. These varieties were chosen based on morphological differences in leaves. The leaf samples were randomly collected from ten plants per variety grown on raised beds in a semi-climate-controlled greenhouse. Hence, a total of 70 plants were utilized for this study. The greenhouse had a temperature of 27±2 °C and 70% relative humidity

2.2 Plant parameters

2.2.1 Leaf Area

It was calculated using ImageJ software and the procedure has been explained under the head Leaf area analysis.

2.2.2 Internode length

It was measured using a scale at 10th internode from the top portion

2.2.3 Stem length

It was measured as the distance from base to apex using a scale.

2.2.4 Stem diameter

It was recorded using a vernier caliper at 5th node of plant from the base.

2.2.5 Bud Diameter

It was measured using vernier caliper at fully turgid state and attainment of full size.

2.2.6 Flower Diameter

It was recorded using vernier caliper across the flower in fully open and expanded stage.

2.3 Image acquisition system

The leaves, stem and flower were arranged on a white chart paper (dimensions) along with a scale and/or coin for reference. A color digital camera model, Sony DSC-HX7V, 10X optical zoom with 16.2 megapixels was located vertically over the chart at a distance of 45 cm. Images were captured using auto mode, without zooming and flash light. The images were saved in. jpeg format for further processing by the software and were transferred into the laptop using a USB cable.

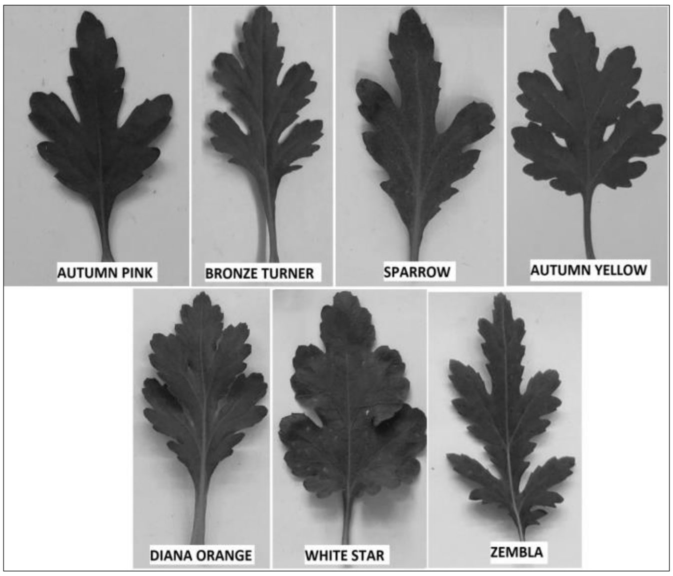

Fig 1: Variations in leaf patterns of chrysanthemum varieties

2.4 Leaf area analysis

Image J version 1.4 is a free public domain, java image processing and analysis program developed at National Institutes of Health, USA. It can be freely downloaded (http://imagej.nih.gov/ij) with relevant plugins and is compatible with all the operating systems. It is well known by researchers dealing with geometric measurement of objects (Schneider *et al*., 2012) [29]. It can enable users in calculation of area and pixel value statistics of user defined selections.

2.5 Steps in image analysis

Open image using ImageJ program. (File → Open → File location)

Fig 2: Home view of Image J software

Take known measurement (eg. 5 cm) of scale/coin using straight line tool. Zoom in if required using + key and zoom out using – key.

Fig 3: Taking the measurement on scale

Go to Analyse → Set Scale → Enter known distance (5cm) → Enter unit of length (cm) → Select global.

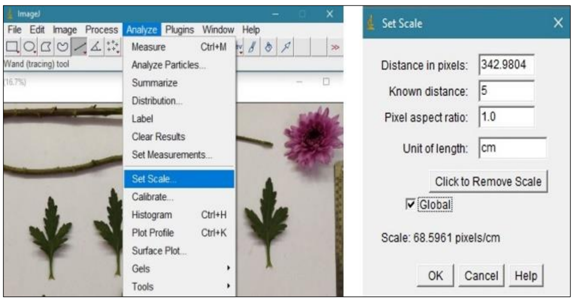

Fig 4: Using the set scale option

Go to Image → Adjust → Colour threshold

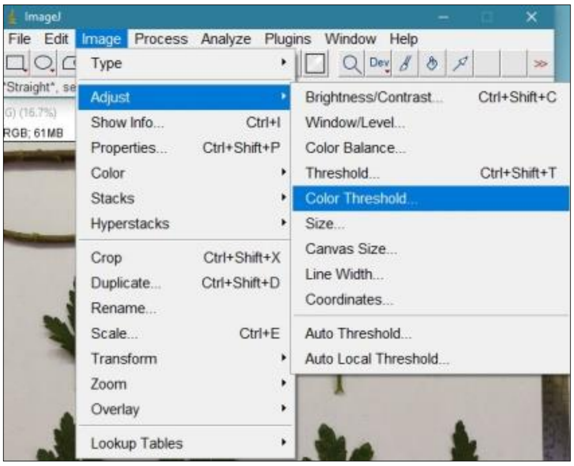

Fig 5: Navigating the color threshold option

Adjust the brightness bars so that leaves are highlighted in colour red and background remains white.

Fig 6: Colour threshold adjustment

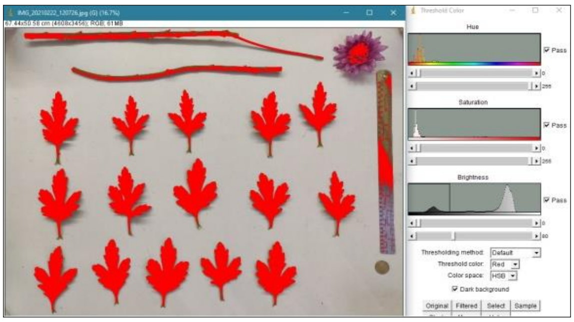

Fig 7: Final image after colour threshold adjustment

Go to Analyse → Tools → ROI Manager

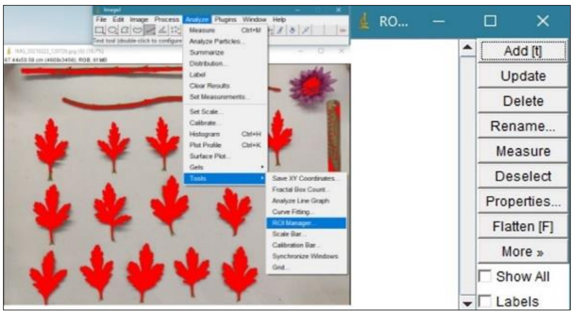

Fig 8: Navigating the ROI Manager

Select the wand tool and click on each leaf followed by clicking on 'add' button in ROI Manager.

Fig 9: Selection of wand tool

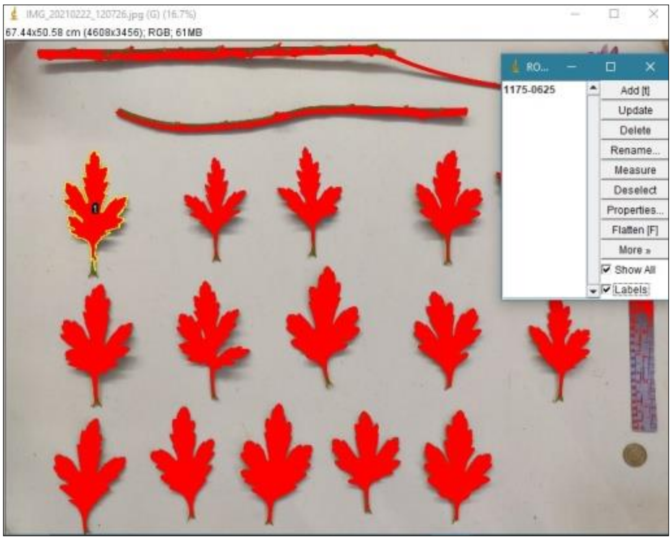

Fig 10: Leaf selection using wand tool

Fig 11: Selected leaves recorded on ROI Manager

Fig 12: Final image after leaf selection

Click on 'Measure' in the ROI Manager.
Go to Results→ Summarize.

Results

File Edit Font Results

	Label	Area	Mean
1		31.371	84.928
2		19.283	84.927
3		21.853	84.976
4		29.041	84.949
5		23.707	84.963
6		35.423	84.945
7		32.747	85.286
8		37.351	84.959
9		29.863	84.895
10		24.654	84.934
11		31.743	84.891
12		30.701	84.905
13		39.177	84.951
14		28.117	84.755
15		35.317	84.917
16	Mean	30.023	84.945
17	SD	5.749	0.108
18	Min	19.283	84.755
19	Max	39.177	85.286

Fig 13: Final measurements

Go to File → Save As to view the data in excel format.

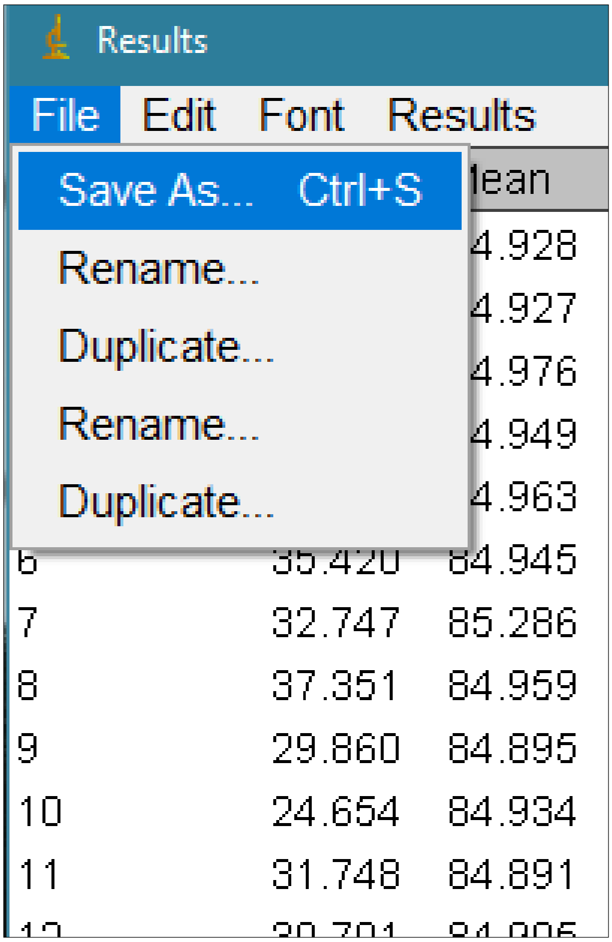

Fig 14: Saving the results

2.6 Statistical analysis
The experiment was set up using randomized block design (RBD). Different parameters were analyzed using OPSTAT software and analysis of variance (ANOVA) was obtained at 0.05% level of significance.

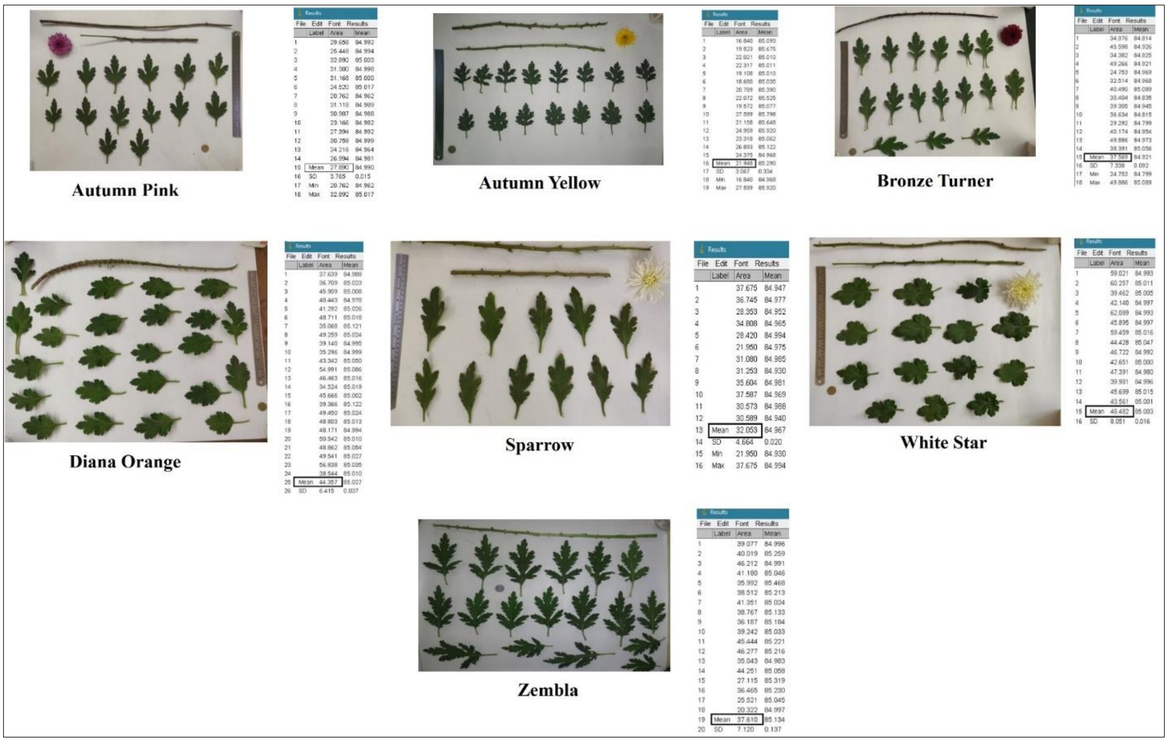

Fig 15: Representative images from each chrysanthemum variety and table generated for leaf area analysis

3. Results and Discussion

Apart from aesthetic and decorative purposes, leaf area is an important feature for chrysanthemum in terms of assessing its productivity, because it determines the photosynthetic assimilates for growth and development. In this experiment, leaf area of different varieties was measured using ImageJ and other characters were studies manually. Table 1 demonstrates average values for leaf area determined through ImageJ software, the standard error and critical difference among the values.

Table 1: Variations in leaf area, intermodal length, stem diameter, stem length, bud size and flower size of different chrysanthemum varieties

Types	Variety name	Leaf Area (cm^2)	Internodal Length (cm)	Stem dial (mm)	Stem length (cm)	Bud size (mm)	Flower size (mm)
Standard type	Zambia (white)	36.20	2.38	3.43	102.91	28.22	104.11
	White Star (white)	48.03	3.15	4.95	108.08	38.80	104.39
Spray type	Autumn Pink (pink)	28.87	1.77	2.48	78.93	16.61	66.17
	Bronze Turner (red)	38.26	2.18	3.10	95.61	22.68	70.85
	Sparrow (white)	31.72	1.83	2.88	90.69	18.76	92.29
	Autumn Yellow (yellow)	22.66	1.25	2.08	57.84	12.28	60.88
	Diana Orange (orange)	45.92	3.14	4.64	97.53	22.89	89.59
S.Em±		1.61	0.06	0.04	1.17	0.61	1.98
CD 0.05		4.59	0.18	0.13	3.33	1.73	5.61

A significant variability was observed. Highest leaf area was observed for White Star (48.03), a standard type variety; which was at par with Diana Orange (45.92), followed by Zembla (36.20), a spray type variety. Minimum leaf area was observed in Autumn Yellow (22.66). These trends were observed in other parameters as well. Internodal length was observed highest in White star (3.15), which was statistically at par with Diana Orange (1.25), followed by Zembla (2.38); while lowest length was seen in cultivar Autumn Yellow (1.25). Among other growth parameters like stem diameter and stem length, highest values were observed in White star (4.95, 108.08) followed by Zembla (3.43, 102.91) while lowest values were seen in Autumn Yellow (2.08). Flowering characteristics like Bud size and flower size were recorded highest in White Star (38.80, 104.39) followed by Zembla (28.22, 104.11) while lowest value was recorded in Autumn Yellow (12.28, 60.88) respectively.

Leaf area is an important aspect in terms of plant productivity and assimilation; since it denotes light interception by photosynthesis and hence can be used to determine vegetative parameters as well (Ferreira *et al*., 2017) [17]. Leaf surface can be used as a tool for future yield and measurement of transpiration losses (Shi *et al*., 2019) [12]. We have used ImageJ software as an inexpensive and efficient tool for measuring leaf area as the method has a small relative error (2.9%) as compared to the graphical method (Patil and Bodhe, 2011) [11].

Our results show that White star, a standard type variety has the highest leaf area, which is correlated with a higher internodal length, plant height, stem diameter, bud size and flower size respectively. This suggests that a higher leaf area can contribute to a higher level of assimilation and food partitioning in plants.

4. Conclusion

In this study, seven chrysanthemum varieties were subjected to analysis using ImageJ program for determining leaf area, while other parameters were calculated on field/manually. There appears to be a positive relationship between leaf area and other growth criteria. These preliminary studies of leaf area provide future scope for developing simulation models of plant growth. The software can be effectively used as a quick and reliable method for yield prediction in advance.

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