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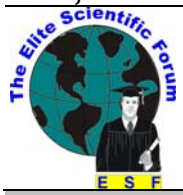

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RESEARCH ARTICLE

Measuring Leaf Area of Winter Cereals by Different Techniques: A Comparison

Shakeel Ahmad[1*], Hakoomat Ali[1], Atique ur Rehman[1], Rana Jahan Zeb Khan[1], Waqas Ahmad[1], Zartash Fatima[1], Ghulam Abbas[1], Muhammad Irfan[1], Hina Ali[2], Muhammad Azam Khan[3], and Mirza Hasanuzzaman[4]
[1]Department of Agronomy, Bahauddin Zakariya University, Multan, Pakistan
[2]The Women University, Multan, Pakistan
[3]Extension Wing, Department of Agriculture, Government of Punjab, Pakistan
[4]Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh

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***Corresponding Author:**
shakeelahmad@bzu.edu.pk

ABSTRACT

Leaf area (LA) is an important parameter to evaluate many traits of plants like canopy, photosynthesis and evapotranspiration. Digital scanners and cameras, in combination with digital image processing software, have replaced older leaf area measuring techniques to determine LA. An experiment was conducted at research area of Department of Agronomy, Bahauddin Zakariya University Multan, Pakistan during 2014-15 to find an easy and precise method of leaf area measurement of selected winter cereals i.e. wheat, barley and oat. Digital image analyzer techniques viz. Easy Leaf Area (ELA) and Image J (IJ) were compared with manual method. The results showed that there was a difference in values recorded by digital analyzer techniques and manual method. The coefficient of determination (R^2) for leaf area measuring techniques viz., manual vs Easy Leaf Area, manual vs Image J, and Easy Leaf Area vs Image J ranged from 0.53 to 0.72, 0.90 to 0.93 and 0.66 to 0.73, respectively for different sowing dates for selected winter cereals. While, similar values for selected winter cereals cultivars were 0.25 to 0.98, 0.74 to 0.97 and 0.49 to 0.93, respectively. However, it is clear from the results that digital analyzer techniques were capable for estimating precisely. The data showed using Image J software was much accurate than Easy Leaf Area. Further, it was found that darkness in image, size of image and quality of image influenced the LA values of winter cereals. However, further application of these digital image analyzers (Easy Leaf Area and Image J) for leaf area measurements may be used in other field crops.

INTRODUCTION

The leaf area index (LAI) is the important variable used to evaluate many processes such as canopy, photosynthesis and evapotranspiration which play an important role in the transformation of energy and mass between the atmosphere and plant canopy (Weiss et al., 2004). The importance of LAI for different agronomic traits in crop production is well understood (Sone et al., 2009; Ahmad et al., 2012, 2015). For estimation of LAI, leaf area (LA) is measured either directly or indirectly. The direct measurement of LA can be destructive or non-destructive; the destructive method requires the collection and transfer of leaf or shoot samples from field to laboratory for further analysis whereas on field linear dimensions of leaves (e.g. length and width) have been widely used to measure LA non-destructively (Bange et al., 2000). Despite the higher accuracy of destructive method the destruction of photosynthetic leaves is undesirable and the method is time consuming (Sanchez-de-Miguel et al., 2011). Montgomery (1911) firstly suggested that leaf area (LA) of plant can be determined from the linear measurement of leaves by using equation A = b x Max length x width, where b is a coefficient (Chanda and Singh, 2002). Leaf area of wheat crop can be measured according to Quarrie and Jones equation where, Leaf area = Length x Breadth x 0.75 (Aldesuquy et al., 2014). Measuring linear dimension of leaves (e.g. length and width) is a well-established method of non-

destructive determination of LA of sunflower crop (Bange et al., 2000). The LAI of different crops depends upon cultivar, different development stages and also on season. The LA is strongly dependent on the local weather conditions and management practices (Jonckheere et al., 2004). Other type of non-destructive direct methods are based on empirical relationship of LA and other variables of the leaf; however, a calibration curve is required before the examination of leaf, but the results are less accurate (Sanchez-de-Miguel et al., 2011).

For indirect measurements of LAI, the plant canopy analyzer, (LAI-200: LI-COR, Lincoln, Nebraska, USA) is widely used apparatus (van Wijk and Williams, 2005). Moreover, Delta-T Devices SunScan meter is another canopy analyzer which use the basic procedure for determining LA from the incoming light above and under the plant canopy. (Potter et al., 1996; Sone et al., 2009).

Many methods have been used to determine LA and several researchers develop procedures that work best with given crop. These methods include mechanical planimeter (Donovan et al., 1958), weight of image (Carleton et al., 1965) and length x width measurement (Donald, 1963). While conceive method include resistance of air flow (Mayland, 1969) and an electric instruments which is for the measurement of non-destructive leaf area, leaf width and leaf length (Wolf et al., 1972). Direct measurement method of LA such as destructive sampling, give more exact LAI values (Potithep et al., 2013).

Digital scanners and cameras, in combination with digital image processing software, have replaced older LA measuring methods to determine leaf area (Abràmoff et al., 2004; Easlon and Bloom, 2014) and a variety of computer based image analyzer, instrument and software are used (Brodny et al., 1986) that are more quick and accurate (Daughtry, 1990). However these software's are only suitable for small plants with few number of leaves (Pandey and Singh, 2011). Image J is the most common software that is used for LA measurements, which use a threshold based pixel count measurement to determining LA however, it may requires serious input and often has faced some difficulties in differentiating leaves from their background (Easlon and Bloom, 2014). Image J program is the innovation of Wayne Rasband of the Research Service Branch, National Institute of Mantel Health, in Brthesda, Mary-land, USA.

Easy Leaf Area is a digital image analyzer software which uses a sequence of thresholding, color ratios and connected component to analyze the LA in a single image within few seconds with little user inputs and the result of leaf area sample are saved in Spreadsheet-ready CSV format (Easlon and Bloom, 2014). The LAI of different crops depends upon cultivar composition and environmental conditions. The LA is strongly dependent on the local weather conditions and management practices (Jonckheere et al., 2004). Thus we used different cultivars on different sowing dates to accommodate the environment and genotype specific changes.

In our experiment, we tried to find out the comparative performance of various leaf area measuring techniques (both destructive and non-destructive); manually and two software's viz. Image J and Easy Leaf Area.

In our experiment, we tried to find the most accurate, simple and time saving technique from destructive and non-destructive methods for winter cereals (wheat, barley and oat) in the field conditions.

MATERIALS AND METHODS

Experimental Site: A field experiment was conducted at Agriculture Research Farm, Department of Agronomy at Bahauddin Zakariya University Multan, Pakistan. The experiment was conducted during winter 2014-15 located at 30.15 °N, 71.30 °E and at an elevation of 122 m from sea level. The soil was characteristically loamy with pH about 8. Annual average temperature was between 25-40°C and annual precipitation was 127 mm. The further detailed description of the experimental site can be found in our previous studies (Ahmad et al., 2012, 2015). The weather data during the growing season of winter cereals are presented in Figure 1.

Land preparation: The land was ploughed with Rotavator to pulverize and to destroy the stubbles of previous crop and weeds. Before sowing winter cereals, land was cultivated 3-4 times to prepare fine seedbed. One irrigation (Rouni) was applied before sowing to provide moisture to seed at initial stage. Crops were sown with manual hand hoe.

Experimental procedure: Three wheat cultivars viz. Sahar-2006, Faisalabad-2008 and Punjab-2011, two cultivars of barley viz. BO 7722, Haider 93 and oat cultivars like viz. CK-01, Ravi were planted at three different sowing dates with interval of 15 days starting from 15th November to 20th December. Net plot size was 3 m x 2 m and the experiment was repeated thrice. Recommended dose of fertilizer was applied at the time of sowing (1/3 of Urea and whole amount of DAP) and remaining (2/3 of Urea) was applied with 1st and 2nd irrigations, respectively. Irrigation was applied after interval of 25 to 30 days. To remove weeds, hoeing was done after 35 days of sowing.

Collection of data: Three plants of wheat, oat and barley were randomly selected and harvested from each experimental unit. Leaves were separated from stem and weighted on electric balance.

LA measurement using manual method: One gram of leaf sample was taken and dimensions of each

sample were measured. For this, piece of flag leaf was placed on a plane page and its length and width were measured from two different positions. Then LA was calculated by following formula; Leaf Area = (length x width) x b

LA measurement using Image J Software: Later on, the weighted sample was placed on a white sheet and the picture was captured with the help of digital camera which was used in Image J for LA measurements. The pictures were transferred to the laptop using bluetooth function for further analysis through Image J software. Complete procedure of Image J is shown in Figure 2.

Following steps were carried by Image J.

Selected picture was placed in Image J (Fig. 2; Step 1). A one cm line was drawn by ruler to see the pixels in one cm. usually one centimeter scale carried 96 to 120 pixels according to picture quality (Fig. 2; Steps 2, 3). Then from toolbar, threshold color was adjusted in such a way that boundary of leaf was appeared red usually the reading of brightness is between 30 and 150 for wheat. It varies from leave to leave and crop to crop. (Fig. 2; Steps 4, 5)

Firstly, we selected wand Tool from the main bar to select tolerance, then 8 connected option for mode was selected and value of "25" was written in Tolerance box and pressed ok (Fig. 2; Step 6). After that it was proceeded as following; (a) Analysis > Tool > ROI Manager; All leaves images were selected one by one and added into ROI manager (Fig. 2; Step 7). An option of "Measure" from the ROI manger was pressed and LA was obtained of all samples (Fig. 2; Step 7). Then for final mean value to result > an option of "Summarize" was pressed to find mean of whole sample. Final value of LA was in cm (Fig. 2; Step 8).

LA measurement using Easy Leaf Area Software: A one gram piece of leaf placed on plain paper along with a red paper piece of 2 x 2 and picture was captured.

Stepwise procedure is shown in Fig. 3 (Steps 1-5). Captured images of leaves were placed into Easy Leaf Area Software. Then clicked "auto-setting" or analyze with current settings. LA value was appeared at top of screen Fig. 3. To open result sheet into excel option of "open output csv file" was clicked (Fig 3; Step 5).

RESULTS

Leaf area recorded by different techniques was significantly varied among treatments. In wheat crop, maximum flag leaf area (119.93 cm^2) was recorded with Easy Leaf Area in a treatment that was shown on 20th November followed by manually recorded leaf area (105.75 cm^2) with sowing date of 20th November. The minimum flag leaf area of wheat (9.73 cm^2) was recorded with Image J on sowing date of 20th December and it was statistically at par those of Easy Leaf Area and manual method (Fig. 4; a, c). Leaf area recorded for

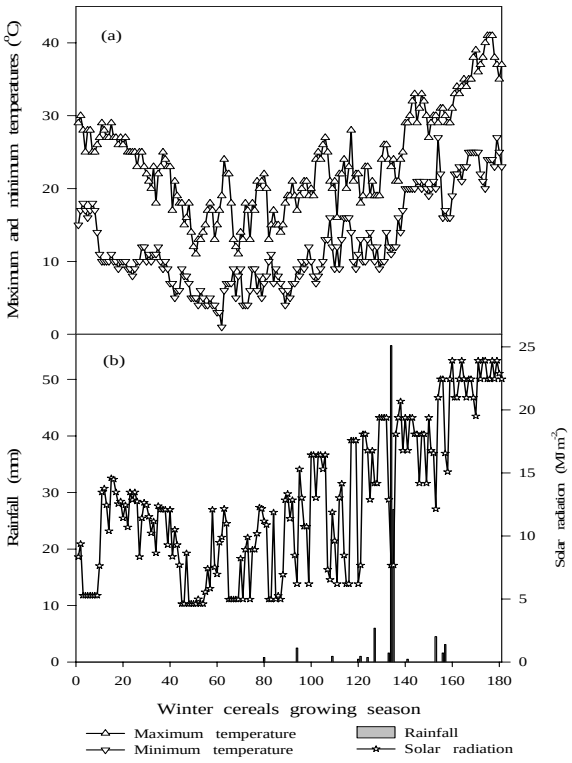
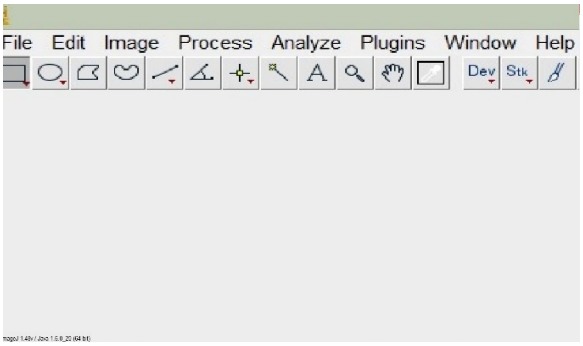


Fig. 1: Daily maximum and minimum temperatures, rainfall and solar radiation during winter cereals growing season at Multan during 2014-15.

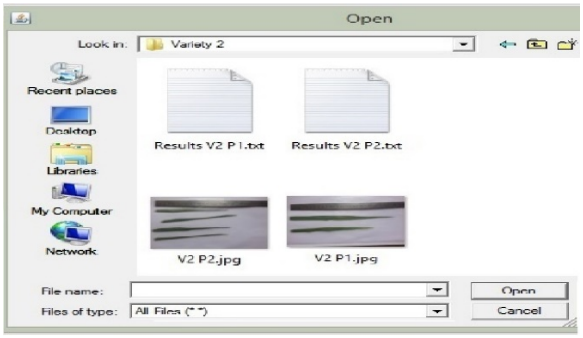
second sowing date i.e. 05th December was more with Easy Leaf Area, however, it was not significant from other methods. Similar trend was recorded for leaf area per plant of wheat (Fig. 4; b, d). Flag leaf area of different wheat cultivars was recorded maximum with manual method (Fig. 4; a, c). Flag leaf area of wheat cultivars recorded by different digital software's was almost similar to each other; however, these software's could not record leaf area as much as recorded with manual method. Similar trend was recorded in leaf area per plant of different wheat cultivars (Fig. 4; b, d). The 1:1 lines for sowing dates and cultivars for wheat crop are presented in Figure 5. The coefficient of determination (R^2) for sowing dates between various LA measuring techniques, viz., manual and Easy Leaf Area, manual and Image J and Easy Leaf Area and Image J were 0.53, 0.93 and 0.66, respectively (Fig. 5; a, b). The similar values for wheat cultivars were 0.25, 0.74 and 0.63, respectively (Fig. 5; c, d).

In barley, LA recorded by different techniques was significant varied among treatments. The maximum flag leaf area of barley (45.24 cm^2) was recorded with manual method in sowing date of 05th December followed by Image J (36.24 cm^2). Similar trend was in crop sown on 20th November, however, crop sown on 20th December recorded statistically similar flag leaf area with different measuring technique (Fig. 4; e, g).

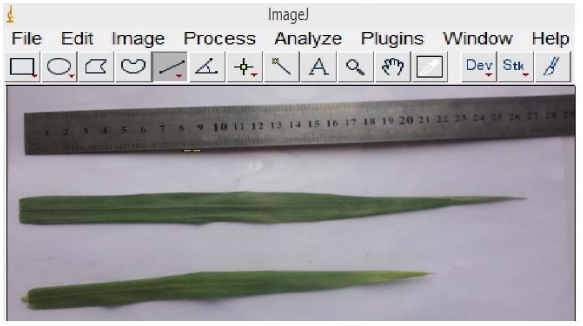
Step 1: Open Image J



Step 2: Select the sample picture

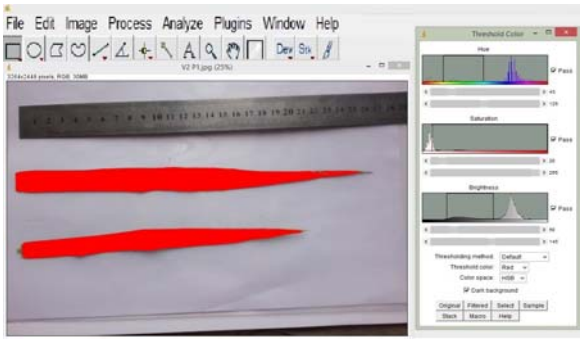


Step 3: Draw a straight line



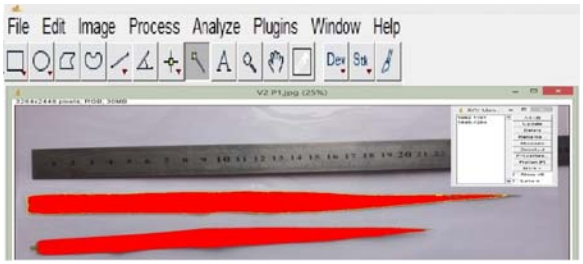
Step 4: Set a Scale

Step 5: Adjust red color with threshold

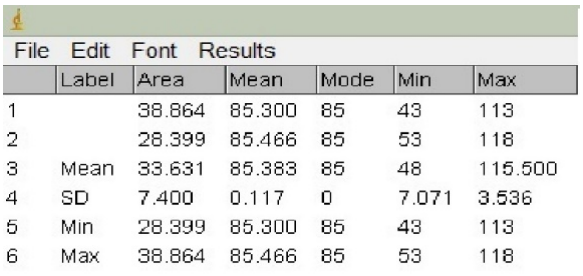


Step 6: Set the Tolerance Value

Step 7: Open ROI Manager and add leaf



Step 8: Result of Image J

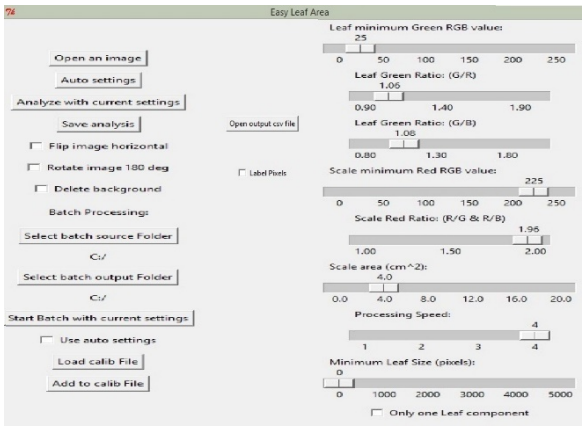


File Edit Font Results

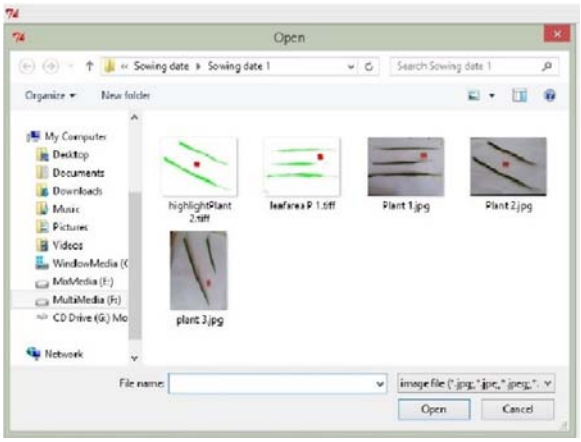
	Label	Area	Mean	Mode	Min	Max
1		38.864	85.300	85	43	113
2		28.399	85.466	85	53	118
3	Mean	33.631	85.383	85	48	115.500
4	SD	7.400	0.117	0	7.071	3.536
5	Min	28.399	85.300	85	43	113
6	Max	38.864	85.466	85	53	118

Fig. 2: Leaf area measuring steps by Image J

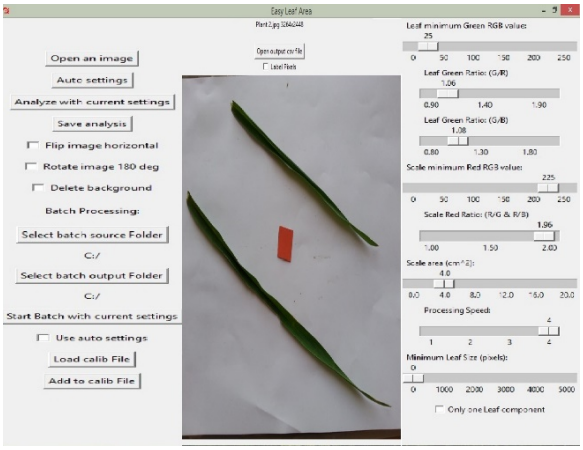
Step 1: Open leaf area software



Step 2: Open the folder

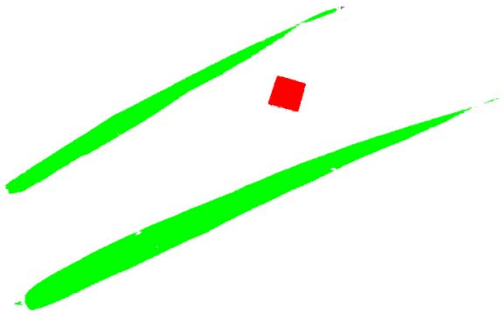


Step 3: Select the image



Step 4: Select Auto Analysis

Step 5: Final image of ELA software



Step 6: Result sheet of ELA

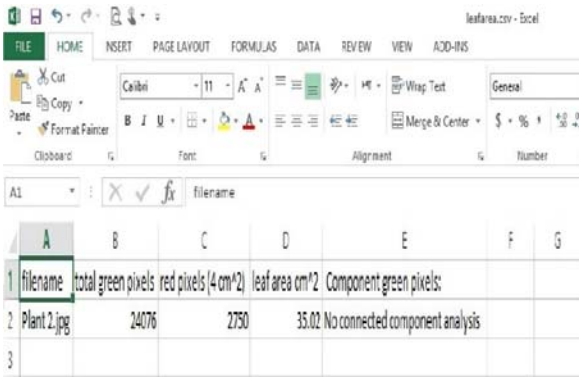


Fig. 3: Leaf area measuring steps by Easy Leaf Area

The LA per plant was recorded maximum with manual method in all sowing dates and it was significantly higher than other techniques. Digital software technique recorded similar leaf area per plant on different sowing dates (Fig. 4; f, h).

In barley cultivars, flag leaf area recorded by manual method was significantly higher than digital software techniques which were at par with each other. Similar trend was recorded in case of leaf area per plant. The 1:1 lines for barley crop sowing dates and cultivars are

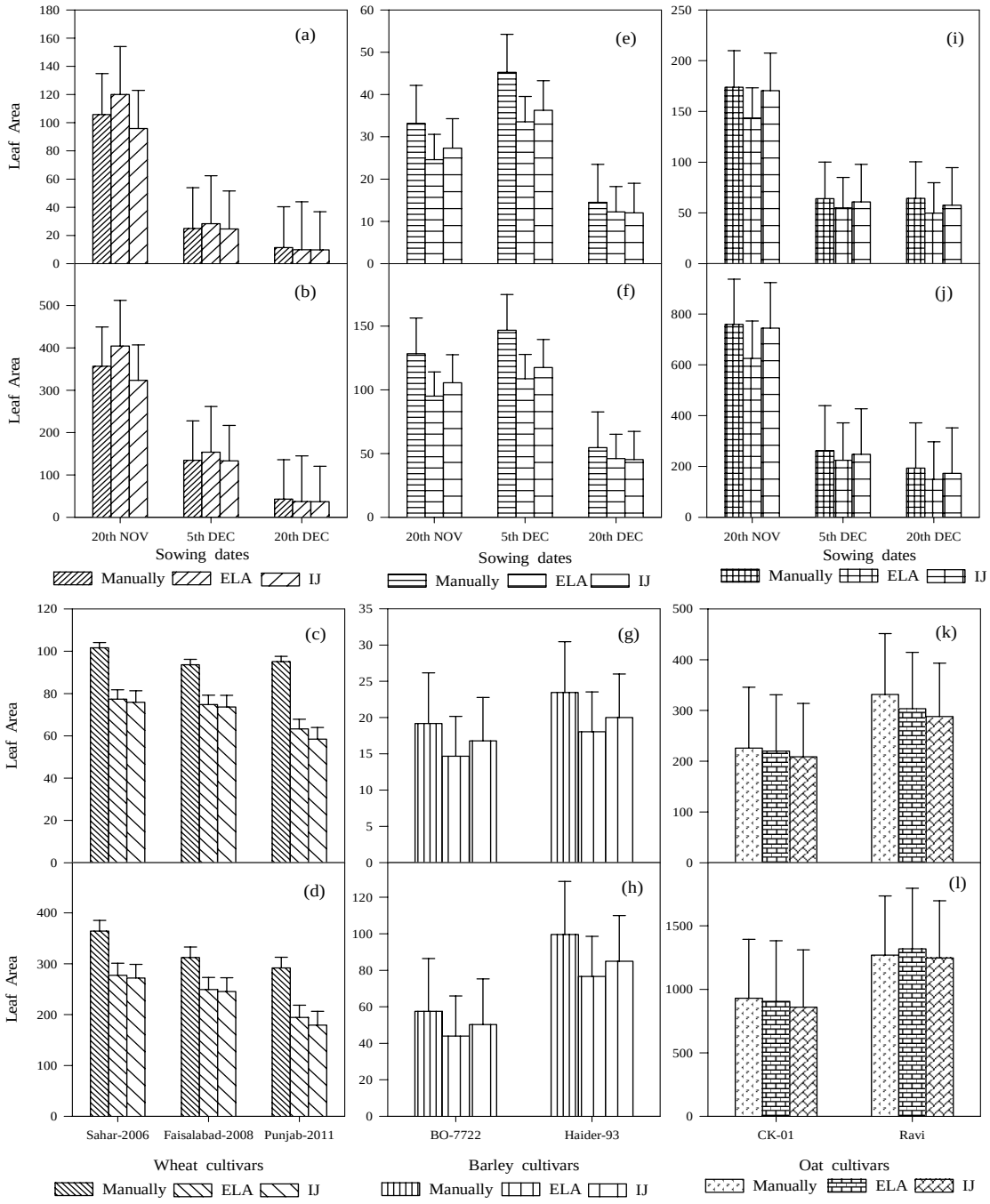


Fig. 4: Wheat, barley and oat flag leaf area and leaf area plant^{-1} as affected by sowing dates for wheat (a, b), barley (e, f) and oat (i, j) and cultivars for wheat (c, d), barley (g, h) and oat (k, l). Bars represent the standard error.

presented in Figure 5. The coefficient of determination (R^2) for sowing dates between various leaf area measuring techniques, viz., manual and Easy Leaf Area, manual and Image J and Easy Leaf Area and Image J were 0.59, 0.96 and 0.72, respectively (Fig. 5; e, f). The similar values for barley cultivars were 0.58, 0.92 and 0.49, respectively (Fig. 5; g, h).

In oat, maximum leaf area recorded by manual method followed by Image J in first sowing date. Easy Leaf Area recorded minimum flag leaf area in 1st sowing date. In crop sown on 5th and 20th December all leaf area measuring technique recorded similar flag leaf area (Fig. 4; i, k). Similar trend was observed recorded in leaf area per plant (Fig. 4; j, l). In oat cultivars, Ravi

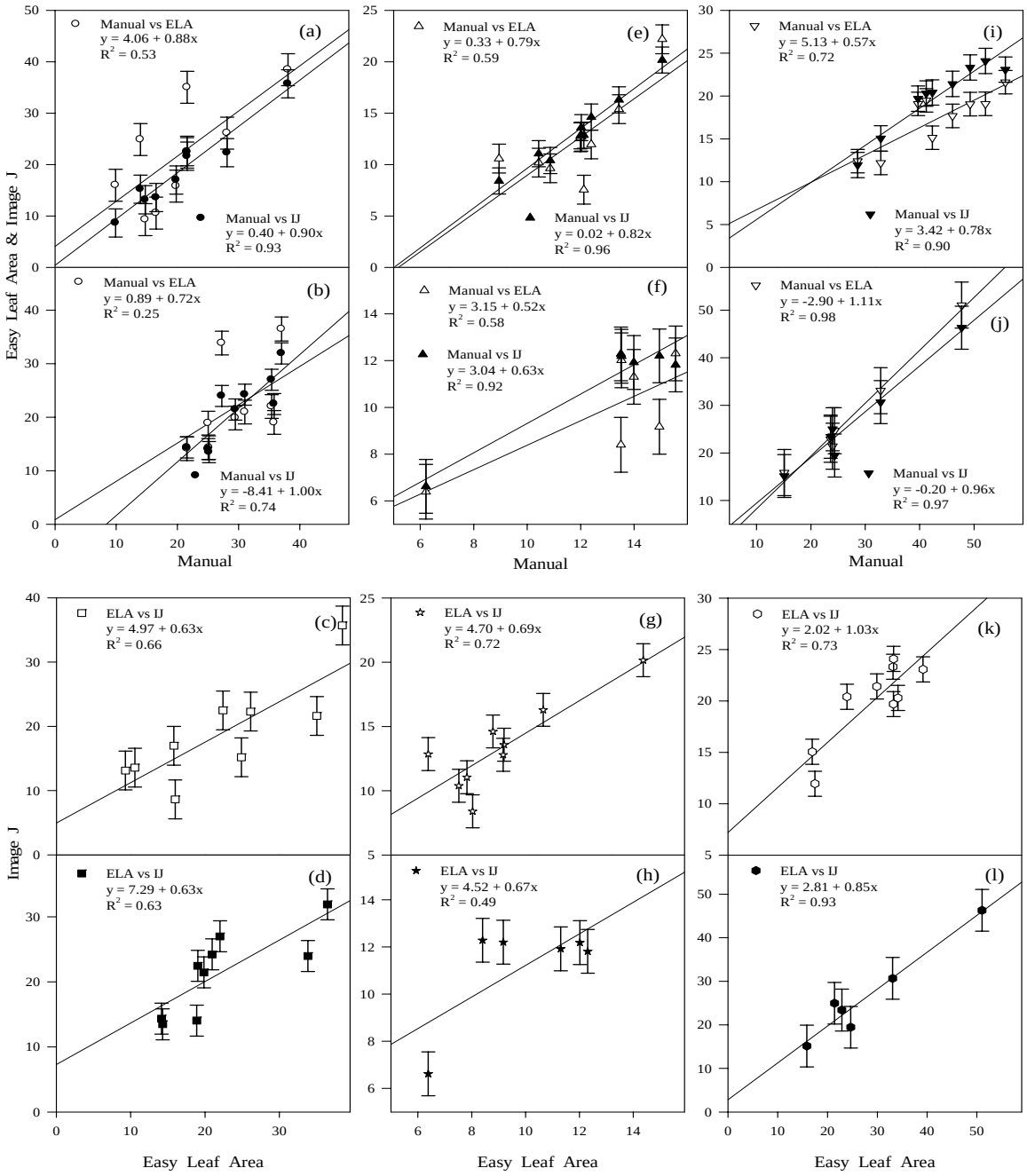


Fig. 5: Wheat (a, b), barley (e, f) and oat (i, j) regression analysis for sowing dates. Wheat (c, d), barley (g, h) and oat (k, l) regression analysis for cultivars. Bars represent the standard error.

recorded more flag leaf area than CK-01 and all leaf area measuring technique were statistically at par with each other. Same trend was recorded in case of leaf area per plant. The 1:1 lines for sowing dates and cultivars of oat crop are presented in Figure 5. The coefficient of determination (R^2) for sowing dates between various leaf area measuring techniques, viz., manual and Easy Leaf Area, manual and Image J and Easy Leaf Area and Image J were 0.72, 0.90 and 0.73, respectively (Fig. 5; i, j). The similar values for oat cultivars were 0.98, 0.97 and 0.93, respectively (Fig. 5; k, l).

DISCUSSION

The results of experiment showed that the digital imaging software Image J and Easy Leaf Area have

good potential for estimating leaf area index of winter cereals. These methods performed well within a different range of leaf size. Destructive method is found time consuming and has less precision as compared to Image analyzer methods. Current measuring techniques in the area under discussion are Image J, Easy Leaf Area and manually method. The non-destructive image analyzer software, Image J took 3 minute and Easy Leaf Area less than 10 seconds, respectively to be analyzed (Easlon and Bloom, 2014). The Image J has higher input as compared to Easy Leaf Area and also detailed result of LA values of a plant. Intensity of light, angle of camera, quality of image are important for a precise result. In Easy Leaf Area, folded or twisted leaf could not analyzed accurately because of less clear image. Software was unable to calculate leaf area of green and red (calibration area) pixels (Kirk et al., 2009). It was observed in our experiment that less chlorophyll content in leaf sample recorded a lower value of leaf area due to yellowish color (Tsuda, 1999). The results of leaf area of selected winter cereals measured by Image J was found more accurate, however, the values recorded were less than manual method (Castillo et al., 2012). The performance of image analyzer methods for measuring leaf area of different crops has been well postulated in different studies (Strachan et al., 2005; Demarez et al., 2008; Liu and Pattey, 2010). However, it is of much importance to use accurate protocols for validation of basic principles of leaf area measurement (Wilhelm et al., 2000).

Digital image analyzer techniques were found easier as well as less time consuming. These were more useful for relatively narrow leaves. It shows their practicality not only for smaller leaves but also their affordability with minimal training. With an easy acquisition of leaf images, it is possible with digital image analyzer techniques to get more rigorous crop informations over a limited space and time (Liu and Pattey, 2010).

Conclusion

Digital image analyzer non-destructive techniques, viz. Image J and Easy Leaf Area are less time consuming and easier to use as compared to manual or destructive method.

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