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Communications in Biometry and Crop Science
Vol. 5, No. 1, 2010, pp. 19–26

International Journal of the Faculty of Agriculture and Biology,
Warsaw University of Life Sciences, Poland

REGULAR ARTICLE

Non-destructive estimation of maize leaf area, fresh weight, and dry weight using leaf length and leaf width

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CITATION: Mokhtarpour, H., Teh, C.B.S., Saleh, G., Selmat, A.B., Asadi, M.E., Kamar, B. (2010). Non-destructive estimation of maize leaf area, fresh weight, and dry weight using leaf length and leaf width. *Communications in Biometry and Crop Science* 5 (1), 19–26.

Received: 5 May 2009, Accepted: 18 May 2010, Published online: 26 May 2010

ABSTRACT

Leaf area and leaf weight measurements are required to calculate several growth indices, which are leaf area index (LAI), net assimilation rate (NAR), specific leaf area (SLA), specific leaf weight (SLW), and leaf area duration (LAD). We developed three predictive equations to estimate leaf area, leaf fresh and dry weight in maize from leaf length and leaf width measurements. A total of 1,314 leaves from different parts of plants at different plant growth stages, different planting densities and different sowing dates were collected in 2008 at the Agricultural Research Center near Gorgan, Golestan, Iran. To evaluate the equations, some goodness of fit indicators used included mean absolute error, root mean square error and index of agreement. This study found strong relationships between leaf length and leaf width and LA, LFW and LDW ($R^2 > 0.85$). Based on the results LA, LFW and LDW of individual maize leaves can be estimated non-destructively by leaf length and leaf width. These equations allow the research workers to make non-destructive or repeat measurements on the same leaves. The general equation to estimate LA, LFW, and LDW was: $\mathrm{Ln}(Y) = a + b\,\mathrm{Ln}(L) + c\,\mathrm{Ln}(W)$.

Key Words: *Zea mays; maize; leaf area; leaf fresh weight; leaf dry weight; non-destructive estimation.*

INTRODUCTION

Leaf area (LA) plays an important role in plant growth analysis. Leaf area and leaf weight measurements are required to calculate several growth indices, which are leaf area index (LAI), net assimilation rate (NAR), specific leaf area (SLA), specific leaf weight (SLW), and leaf area duration (LAD) (Gardner et al., 1985). There are various methodological approaches to measure plant leaf area. Direct measurement of leaf area is usually time consuming and labor intensive and this action usually causes canopy damage. But leaf area can be estimated non-destructively by using mathematical formulae, which only require simple measurements of the leaf lamina. Potdar and Pawar (1991) evaluated non-destructive leaf area estimation in banana (*Musa acuminata* Colla.) and showed a strong relationship between leaf area and various combinations of leaf length (L) and leaf width (W). Pekson (2007) also showed that there was a high correlation between leaf area and a combination of lamina length (L) and lamina width (W) in *Vicia faba* L. Serdar and Demirsoy (2006) developed a mathematical equation to estimate leaf area in chestnut (*Castanea* sp.) by measuring leaf length and leaf width and calculated different combination of them. Their result showed that there was a strong relationship between estimated leaf area and actual leaf area (R^2 = 0.99). Cho et al. (2007) found that estimation of individual LA, leaf fresh weight (LFW) and LDW in hydroponically grown cucumbers (*Cucumis sativus* L.) can be done with high accuracy using leaf length leaf width and leaf chlorophyll value (R^2 = 0.98, R^2 = 0.96, R^2 = 0.96 respectively).

Montgomery (1911), cited in McKee (1964), Pearce et al. (1975), and Dwyer and Stewart (1986), reported a general equation to estimate individual leaf area of maize (*Zea mays* L.):

Leaf area = L × W × A

where LA, L, W, and A are leaf area, leaf length, leaf maximum width and a constant (A = 0.75), respectively. Other researchers obtained A values between 0.72 and 0.79, for example 0.72 (Keating and Wafula, 1992), 0.73 (McKee, 1964; Dwyer and Stewart, 1986; Stewart and Dwyer, 1999), and 0.79 (Birch et al., 1998). McKee (1964) proposed that total leaf area may be estimated by multiplying the sum of the lengths of all leaves on a plant by 6.67. Pearce et al. (1975) proposed multiplying the area of the eighth leaf from the top by 9.39. Elings (2000) showed that if the total number of leaves of tropical maize and area of the largest leaf are known, total plant leaf area can be estimated directly, making use of the fact that the area of the largest leaf relative to total plant leaf area is constant, and that this constant is linearly related to total leaf number.

The objectives of the current study were to develop equations to estimate leaf area (LA), leaf fresh weight (LFW), and leaf dry weight (LDW) of maize from leaf length and width. The accuracy of the equations was tested on maize at various planting densities, plant growth stages and planting dates. The equation developed to estimate leaf area was tested against equations from other studies.

MATERIALS AND METHODS

To evaluate non-destructive LA, LFW, and LDW estimation for four maize planting dates, three planting densities, and four plant growth stages, all leaves from 144 plants were selected from an experiment conducted at the Agricultural Research Center near Gorgan, Golestan, Iran (36° 53' N, 54° 21' E) in 2008 to evaluate the effect of planting date and density on yield and yield components of maize. Planting dates included 19 April, 4 May, 19 May and 3 June. Plant densities were 45,000, 65,000 and 85,000 plants ha^{-1} and plant growth stages were the 5-leaf stage, when the 6th leaf appears but it is too small to measure (V3), 8-leaf stage, when the 9th leaf appears but it is too small to measure (V6), tassel appearance stage (flag leaf was not counted) and milk stage. Each plot contained four rows, 12 m in length. The distance between rows was 75 cm and planting densities were changed with changing distance between plants row^{-1}. Distance between plants row^{-1} was 30, 20, 15.7 cm for planting densities 45000, 65000, and 85000, plant ha^{-1}, respectively. The experiments were planted manually in four complete blocks. Three seeds were planted in each hole and then thinned to one plant at the 2-leaf stage so survived plants were same with intended planting densities. Experiment was conducted without any water and nutrient limitation. Soil water was kept over 50% of field capacity during the growing season by furrow irrigation. Fertilizers were applied based on soil test results. A broadcast application of 60-45-100 kg ha^{-1} (N-P-K) was incorporated into the seedbed. Additional 100 kg N ha^{-1} was applied as side dressing at 5 and 9-leaf stage (50 kg ha^{-1} at each stage). Weeds and insects were adequately controlled during the growing seasons. In order to measure leaf length, leaf width, and leaf area in each treatment, at each harvest, three plants were cut at ground level from three replications considering border effects (one plant from each replication). Leaf length and maximum leaf width of all leaves on each plant were measured manually, and the leaf area of each leaf was then measured using the Area Measurement System (Delta-T Devices Ltd, Cambridge, UK). Leaf fresh weight of each leaf was measured immediately after it was removed from the stalk. Leaves were dried to a constant weight at 75°C for about three days.

A total of 1,368 leaves from 114 plants (four planting dates × three plant densities × four plant growth stages × three plants in each sampling) were collected for this study. The data from 54 leaves were not recorded due to mechanical damages to leaves during sampling time in different treatments and partial leaf senescence after anthesis. So a total of 1,314 leaves were used in this study. The data from 855 leaves (approximately two third of total leaves) were selected at random and pooled without considering planting date, plant density and plant growth stages. Curve-fitting software, Table Curve 3D® (version 4; Systat, Inc, Chicago, IL, USA), was used to fit the best equation for estimation of LA, LFW, LDW. To evaluate the accuracy of the equations data from the other 459 leaves (approximately one third of total leaves) were used to test whether the equations developed could predict LA, LFW, and LDW with high accuracy at different planting dates, plant densities and plant growth stages; Model validity was tested using three goodness of fit indicators, the mean absolute error (MAE), the root mean square error (RMSE) (Kobayashi and Salam, 2000), and the index of agreement (*d*) (Willmott et al., 1985). Their formulae are as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}}$$

$$MAE = \frac{\sum_{i=1}^{N}|y_i - \hat{y}_i|}{N}$$

$$d = 1 - \frac{\sum_{i=1}^{N}|y_i - \hat{y}_i|}{\sum_{i=1}^{N}\left(|\hat{y}_i - \overline{y}| + |y_i - \overline{y}|\right)}$$

where y_i and $\hat{y}_i$ are the observed and predicted y values, respectively, and $\overline{y}$ is the mean of the entire N of observed y values. Low values of RMSE and MAE illustrate high accuracy whereas high d indicates high accuracy.

RESULTS AND DISCUSSION

EQUATION DEVELOPMENT

We used three criteria to select the best-fitting function for the data in the 850-leaf training set: (1) the function with a close degree of fit to the data set as indicated by a high R^2: (2) the function shows no overall estimation or prediction bias; and (3) the function is parsimonious (Burnham and Anderson, 2002). The latter criterion means the function fits the data set closely with the least number of function parameters. A parsimonious function avoids "over-fitting" a given data set. The danger of over-fitting is the function describes random error instead of the underlying relationship. Such a function will fit the training set almost perfectly while having little predictive value, being too sensitive to fluctuations in the data set (see Hunts and Parsons, 1977).

Both leaf length and leaf width had a strong relationship with leaf area and LFW with high coefficients of determination ($R^2 \geq 0.97$) and index of agreement $d \geq 0.91$ (Table 1). Leaf dry weight (LDW) was not estimated as accurately as leaf area and LFW ($R^2 = 0.88$, $d = 0.834$). The lower estimated accuracy of LDW could be due to differences in the specific leaf area (SLA) at different plant growth stages.

Table 1. Equations developed for estimating maize leaf area, leaf fresh weight and leaf dry weight, using a training set of 853 leaves, which were drawn from a total of 1314 leaves representing four seeding dates, three plant densities, four growth stages and three plants for each combination of these factors. All variables in the models above are significant at $P = 0.01$.

Response variable	Intercept		Ln(leaf length)		Ln(leaf width)			
Ln (leaf trait)	Estimate	SE	Estimate	SE	Estimate	SE	R^2	P-value
Area (LA), cm^2	-0.990	0.058	1.231	0.021	0.854	0.019	0.98	<0.001
Fresh weight (LFW)	-5.469	0.078	1.418	0.028	0.829	0.026	0.97	<0.001
Leaf Dry weight (LDW)	-8.704	0.234	1.171	0.083	2.079	0.077	0.87	<0.001

EQUATION EVALUATION

To evaluate the accuracy of the equations, three goodness-of-fit-indicators (RMSE, MAE, and *d*) were determined for treatment factors plant density, plant growth stage, and planting date using a validation set of 459 leaves that were not part of the training data set. The highest and lowest values for RMSE, MAE, and *d* for leaf area were found among growth stages (Table 2). The index of agreement exceeded 0.90 in all cases.

According to Bland and Altman (1986), cited in Peksen (2007), lack of agreement between predicted values and measured values were evaluated by calculating the relative bias, estimated by the mean of differences (MD) and the standard deviation of a difference (SD). When the differences are distributed normally, 97 % of the differences should be between MD ± (3 × SD). There was a linear relationship in the validation data set of 459 leaves between measured leaf area and leaf area predicted on the basis of the equation developed from the training data set of 855 leaves (Fig. 1 top row, left panel; Table 2) with an index of agreement of 0.93. Ninety-nine percent of the differences between measured and predicted leaf areas were within ± 3 SD of the mean difference (Fig. 1 top row, right panel), which is in agreement with normal distribution theory. However, the graph also indicates that the difference increased with increasing leaf area.

The prediction of leaf fresh weight with the prediction formula developed in the first step worked quite well with *d*-values ≥ 0.88 (Table 3). The graph of measured vs. predicted based on pooled data of 459 leaves in the validation set indicates that the actual relationship may be curvilinear (Fig. 1 middle row, left panel) and assessment that is supported by the plot of differences between measured and predicted values (Fig. 1 middle row, right panel).

The index of agreement between measured and predicted leaf dry weight was lower than the index for leaf area and leaf fresh weight. It ranged from $d = 0.71$ at the grain filling stage to $d = 0.84$ for the first and third planting date (Table 4). This is borne out by the graph, which shows a considerable spread around the predicted values (Fig. 1 bottom row, left panel). All differences between observed and predicted fell within ± 3 SD of the mean difference (Fig. 1 bottom row, right panel).

Lastly, we compared our prediction formula against the commonly used equation by Montgomery (1911), mentioned in the introduction. The best estimate for constant A was 0.754. Using the value, our newly developed prediction formula resulted in a lower RMSE (35.6 vs. 38.5), a slightly lower MAE (26.1 vs. 27.2), and an almost identical index of agreement (*d* = 0.934 vs. 0.929).

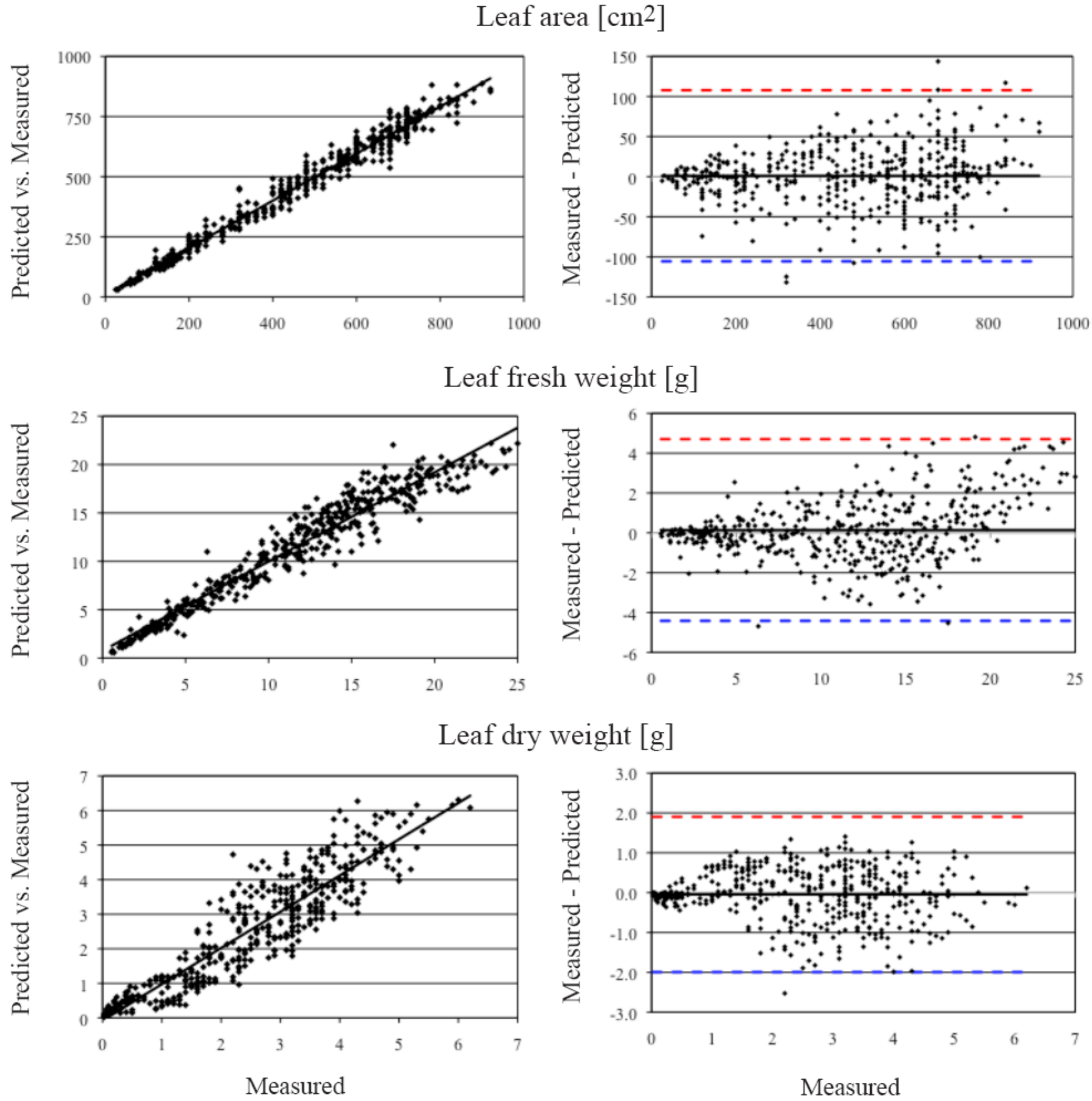

Figure 1. Relationship between values predicted from the regression equations and measured values for leaf area, leaf fresh weight and leaf dry weight for the 459 leaves not in the training set. The left column of panels depicts the actual relationship based on predictions from equations given in Table 1, where the solid line represents the linear regression of Y on X. The right column of panels depicts the difference between measured and predicted values, where the top (red) hashed line represents the mean + 3 SD, and the bottom (blue) hashed line the mean – 3 SD.

Table 2. Evaluation of the equation developed to predict maize leaf area. The test data set consisted of 459 leaves that were not part of the training data set.

Treatment	Root mean square error (RMSE)	Mean absolute error (MAE)	Index of agreement (*d*)	Number of leaves
		cm^2		
Planting density, plants ha^{-1}				
45000	21.9	28.7	0.92	163
65000	22.6	29.1	0.92	149
85000	22.3	30.2	0.92	147
Plant growth stage				
5-leaf	4.0	8.9	0.91	51
8-leaf	12.6	21.8	0.94	78
Tasseling	25.7	36.9	0.90	128
Milk stage	27.4	31.1	0.91	202
Planting date				
19 April	21.9	32.3	0.93	117
04 May	18.3	28.1	0.93	111
19 May	18.4	25.9	0.94	115
03 June	17.7	26.8	0.92	116
All data				
	35.6	26.1	0.93	459

Table 3. Evaluation of the equation developed to predict maize leaf fresh weight. The test data set consisted of 459 leaves that were not part of the training data set.

Treatment	Root mean square error (RMSE)	Mean absolute error (MAE)	Index of agreement (*d*)	Number of leaves
		g		
Planting density, plants ha^{-1}				
45000	0.88	1.07	0.91	163
65000	0.72	1.00	0.91	149
85000	0.67	0.87	0.93	147
Plant growth stage				
5-leaf	0.12	0.27	0.89	51
8-leaf	0.71	1.32	0.88	78
Tasseling	0.72	1.06	0.88	128
Milk stage	1.12	1.31	0.88	202
Planting date				
19 April	0.68	1.02	0.91	117
04 May	0.53	0.81	0.93	111
19 May	0.69	1.01	0.92	115
03 June	0.77	1.15	0.90	116
All data				
	1.52	1.12	91.00	459

Table 4. Evaluation of the equation developed to predict maize leaf dry weight. The test data set consisted of 459 leaves that were not part of the training data set.

Treatment	Root mean square error (RMSE)	Mean absolute error (MAE)	Index of agreement (*d*)	Number of leaves
		g		
Planting density, plants ha^{-1}				
45000	0.458	0.211	0.83	163
65000	0.374	0.158	0.82	149
85000	0.337	0.151	0.83	147
Plant growth stage				
5-leaf	0.053	0.012	0.72	51
8-leaf	0.352	0.106	0.81	78
Tasseling	0.332	0.137	0.78	128
Milk stage	0.431	0.242	0.71	202
Planting date				
19 April	0.295	0.111	0.84	117
04 May	0.328	0.126	0.83	111
19 May	0.391	0.143	0.84	115
03 June	0.345	0.131	0.80	116
All data				
	0.65	0.49	0.83	459

ACKNOWLEDGEMENTS

We thank the Agricultural and Natural Resources Research Center, Golestan, Iran for financial support. Our especial thanks go to Mr. Feizbaksh and Mr. Hydari, Department of Seed and Plant Improvement, Agricultural and Natural Resources Research Center, Golestan, Iran for their assistance in conducting the experiments and data collection.

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