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A Rapid and Non-Destructive Method to Determine the Leaflet, Trifoliate and Total Leaf Area of Soybean

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ABSTRACT

The importance of rapid, non-destructive, and accurate measurements of leaf area for agronomic and physiological studies is well known. Several mathematical formulas have been derived for estimating leaf areas for numerous crops, but there is little information available for soybean (*Glycine max* L.). This study aimed to develop prediction equations for estimating leaflet, trifoliate and total leaf areas using maximum length, L (cm), width, W (cm), length and width product, LW (cm) and green leaf dry matter, DM (g) of soybean leaves. For this purpose, an experiment was conducted using three cultivars of soybean ('Dpx', 'Sahar' and 'Williams'), in 2009-2010, in the Faculty of Agronomy, Gorgan, Iran. During the growing season, leaves of randomly selected soybean plants were collected. Leaf area was measured with a digital leaf area meter, related dry matter also was weighed, leaf dimensions were determined with a ruler, too. Statistical analyses of soybean leaf areas were divided into three levels: leaflet, trifoliate and total leaf area. At each level, the predictive abilities of three regression equations (linear, power and binomial) were compared, with different independent variables for each equation. Our data indicate, however, that considerable savings of time, with little loss of predictive ability, could be possible by measuring only W or LW in each instance. In general, these analyses indicated that a single regression equation could be used at each level. Our findings revealed that pooled-based models (without respect to cultivar) are reliable for estimating leaflet, trifoliate and total leaf area. Researchers can use these models readily and without any inconvenience to save time and costs, especially where there is a lack of related equipment to measure leaf area.

Keywords: green leaf dry matter, length, models, width

INTRODUCTION

Leaves are the most important photosynthetic organ of the plant. Estimation of leaf area (LA) is an essential component of plant growth analysis and evapotranspirational studies. LA is a determinant factor in radiation interception, photosynthesis, biomass accumulation, transpiration and energy transfer by crop canopies. It is also important with respect to crop-weed competition and soil erosion (Jonckheere *et al*. 2004; Akram-Ghaderi and Soltani 2007). LA is useful in the analysis of canopy architecture as it allows determination of leaf area index (LAI). Therefore, LA is measured in many different studies and its accurate measurement is necessary for understanding crop responses to experimental treatments. Although many methods are available for LA measurements, such as graded standards, these methods often are time consuming and laborious. Although sophisticated electronic instruments provide accurate and fast LA measurement, they are expensive, especially in developing countries. Hence need to develop economically cheaper and technically easier but sound methods are needed for LA measurement (Korva and Forbes 1997). Nondestructive methods allow the replication of measurements during the growth period, reducing some of the experimental variability associated to destructive sampling procedures (NeSmith 1992). They are very useful in studies of plant activity, which require a nondestructive method of measuring LA (Wendt 1967) and also when the number of available plants is limited. The use of regression equations to estimate LA is a nondestructive, simple, quick, accurate, reliable and inexpensive. The usual procedure of this method involves measuring maximum length (L), width (W) and area of a sample of leaves and then calculating the several possible regression coefficients, or leaf factors, to estimate areas of subsequent samples (Wiersma and Bailey 1975). Accurate and simple mathematical models eliminate the need for leaf area meters or time-consuming, geometric reconstructions (Gamiely *et al.* 1991). Montgomery (1911) first suggested that LA of a plant can be calculated from linear measurement of leaves. Such a mathematical equation for estimating LA reduces sampling effort and cost, may increase precision where samples of leaf size are difficult to handle. There are a number of prediction equations using leaf dimension measurements (L and W) for leaf area measurement of different crops and also, there are number of leaf area prediction models based on individual leaves, leaf weight or total aboveground biomass (**Table 1**). Leaves are formed in a characteristic pattern for each species, creating a specific leaf shape (Sinha 1999). Therefore, prediction models must be determined for each species, and for cultivars of a given species which presents different leaf shapes. Soybean, as the most important summer crop in Golestan province, North of Iran, has a valuable situation in cropping patterns which is cultivated just after wheat harvesting. A wide rang of studies with different aims are underway in universities and research centers in which, in most cases, LAI measurement is needed. But LA measurement is time-consuming and laborious and needs special equipment, which is expensive, thus it seems that a simple measurement based on simple methods is needed to help researchers in this case. However, available information for non-destructive prediction of soybean (*Glycine max* L.) LA is scarce (Wiersma and Bailey 1975). The purpose of this study, therefore, was to develop prediction equations for estimating leaflet, trifoliate and total leaf areas by using L, W, length and width product (LW) and green leaf dry matter (DM) of soybean leaves.

Received: 30 March, 2010. Accepted: 18 May, 2010.

Original Research Paper

Table 1 Mathematical relationships between leaf area with vegetative traits in different crops.

Source	Published relationships	Crops	Variable
Bhatt and Chanda 2003	$LA = 11.98 + 0.06 \times LW$	Common bean (*Phaseolus vulgaris* L.)	LW
	$LA = 0.11 + 0.88 \times (L + W)$		L+W
Peksen 2007	$LA = 0.919 + 0.682 \times LW$	Faba bean (*Vicia faba* L.)	LW
Jesus and Vale 2001	$LA = 2.137 \times L^{1.964} - 2.70$	Common bean (*Phaseolus vulgaris* L.)	L
Kathirvelan and Kalaiselvan 2007	$LA = 0.895 \times L^{1.08} \times W^{0.76}$	Groundnut (*Arachis hypogaea* L.)	L, W
Kumar 2009	$LA = 191.33 \exp^{L\times0.0037}$	Saffron (*Crocus sativus* L.)	L
Palaniswamy and Gomez 1974	$LA = 0.74 \times LW$	Rice (*Oryza sativa* L.)	LW
Tsialtas and Maslaris 2005	$LA = 18.92 \times W - 80.54$	Sugar beet (*Beta vulgaris* L.)	W
	$LA = 16.37 \times L - 174.1$		L
Tsialtas and Maslaris 2008a	$LA = 0.243 \times L^{2.28}$	Sunflower (*Helianthus annuus* L.)	L
	$LA = 2.15 \times W^{1.82}$		W
Shin *et al.* 1981	$LA = 0.741 \times LW$	Sorghum (*Sorghum bicolour* L.)	LW
	$LA = 0.083 \times L^2$		L
Tsialtas *et al.* 2008	$LA = 0.587 \times LW$	Grapevine (*Vitis vinifera* L.)	LW
Tsialtas and Maslaris 2008b	$LA = 0.003 \times LW^2 - 1.03 \times LW + 296.8$	Sugar beet (*Beta vulgaris* L.)	LW
Bange *et al.* 2000	$LA = - 11.2 \times L + 12.3 \times W + 0.66 \times LW$	Sunflower (*Helianthus annuus* L.)	L, W
Rouphael *et al.* 2007	$LA = 6.72 + 0.65 \times W^2$	Sunflower (*Helianthus annuus* L.)	W
Lu *et al.* 2004	$LA = 0.72 \times LW$	Taro (*Colocasia esculenta* L.)	LW
Kandiannan *et al.* 2009	$LA = - 0.014 + 0.66 \times LW$	Ginger (*Zingiber officinale* Ros.)	LW
Tsialtas and Maslaris 2008c	$LA = 43.4 \times LDW^2 - 10.7 \times LDW + 118.3$	Sugar beet (*Beta vulgaris* L.)	LDW
Ma *et al.* 1992	$LA = 0.023 \times LDW^{0.97}$	Peanut (*Arachis hypogaea* L.)	LDW
	$LA = 0.02 \times TBM^{0.85}$		TBM
Payne *et al.* 1991	$LA = 162.8 \times LDW^{0.687}$	Pearl millet (*Panicum milieaceum* L.)	LDW
Sharratt and Baker 1986	$LA = 28.7 \times LDW^{0.993}$	Alfalfa (*Medicaco sativa* L.)	LDW
	$LA = 10.7 \times TBM^{0.992}$		TBM
Ramos *et al.* 1983	$LA = 6.85 + 244.86 \times LDW$	Barley (*Hordeum vulgare* L.)	LDW
Akram-Ghaderi and Soltani 2007	$LA = 125.3 \times LDW^{1.078}$	Cotton (*Gossypium hirsutum* L.)	LDW
Rahemi *et al.* 2006	$LA = 156.02 \times LDW^{1.16}$	Chickpea (*Cicer arietinum* L.)	LDW
Shin and Snyder 1984	$LA = 223 \times LDW$	Taro (*Colocasia esculenta* L.)	LDW

L, length; LA, leaf area; LDW, leaf dry weight; LW: length and width product; TBM, total biomass matter; W, width

MATERIALS AND METHODS

A field experiment was conducted in the 2009-2010 growing season at the Research Field of Gorgan University of Agricultural Science and Natural Resource located at 37° 45′ N, 54° 30′ E, and 100 m asl, Iran. The field soil is silty clay loam and the climate is temperate sub-humid. Three soybean cultivars as the main cultivating varieties in North of Iran (‘Dpx’, ‘Sahar’ and ‘Williams’) were planted (4 July) and standard crop management practices were followed. Therefore, during experiment there was no effect water deficit stress, the effect of diseases, pasts and weeds were minimal, too. Plants were sampled randomly at the R1 to R6 growth stages (Fehr and Caviness 1977) and were taken to the laboratory and their leaves were cut and terminal leaflet L and W of each leaf were measured with a simple ruler. Leaflet L was measured to the nearest mm from lamina tip to the point of intersection of the lamina and the petiole, along the midrib of the lamina, while leaflet W was measured from end-to-end between the widest lobes of the lamina perpendicular to the lamina midrib (**Fig. 1**). Actual area (AA) of all leaflets and trifoliate separately was measured with a digital area meter per plant (DELTA-T, Co. Durham, UK). Means, standard deviations, minimum and maximum values of the leaflet L, W and LA for each soybean cultivar and pooled data are shown in **Table 2**.

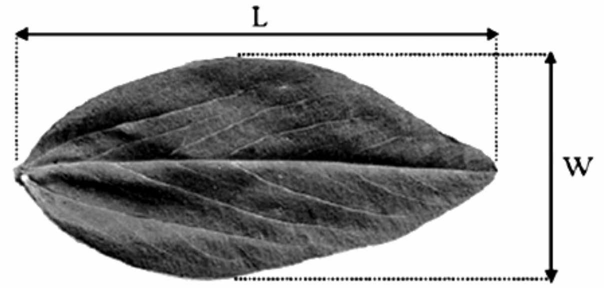

Fig. 1 Measured parts of the soybean leaflet to determine the leaflet length (**L**) and width (**W**).

The leaf samples of each plant were then oven dried at 70°C till consecutive constant weights. Sampled leaves represented the full spectrum of measurable leaf sizes presented at the developmental stage, and did not present any damage and deformation caused by diseases, insects or other factors. This work examined the relationship between leaflet, trifoliate and total leaf areas and length and width dimensions and dry weight in an attempt to identify appropriate functions for use in models estimating leaf area of soybean. The relationship between leaflet and trifoliate area as a dependant variable and L, W and LW as independent variables was determined using regression analysis on data of 1150 leaves. Also, the relationship between plant the total leaf area as a dependant variable and summed Length (ΣL), summed Width (ΣW), summed length and width product (ΣLW) and DM as independent variables was determined using regression analysis on data of 120 plants. Coefficients of determination (R^2) were calculated and the equation with the highest R^2 was used in the final estimations. The linear, polynomial and power Functions were developed through SAS software (SAS Institute 1992) and Excel

Table 2 Characteristics the terminal leaflet different soybean cultivars (Data are from pool all the different growth stages (R1 to R6) for each cultivar).

Cultivars	N	L[a]			W[a]			LA[b]		
		Me ± SD	Max	Min	Me ± SD	Max	Min	Me ± SD	Max	Min
Dpx	451	10.3 ± 2.9 a	17.7	2.2	5.9 ± 2.3 a	11.5	1.3	45.2 ± 28.3 a	134.1	0.6
Sahar	455	8.7 ± 2.6 a	16.0	3.1	4.6 ± 1.7 a	10.5	1.5	30.1 ± 18.8 a	109.8	1.9
Williams	244	8.9 ± 2.8 a	15.5	3.0	5.5 ± 1.9 a	10.0	1.3	37.4 ± 23.6 a	107.3	3.0
Pooled data	1150	9.4 ± 2.9 a	17.7	2.2	5.3 ± 2.1 a	11.5	1.3	37.5 ± 24.8 a	134.1	0.6

LA: leaf area, L: length, MAX: maximum, Me: mean, MIN: minimum N: number, SD: standard deviation, W: width
[a] centimeter, [b] centimeter^2

worksheet.

The estimated LA was determined by fitting the equations. Then estimated and measured leaf areas were compared by testing the significance of regression equation and degree of goodness of fit R^2 between estimated and observed values. The model with lower root mean square error (RMSE), higher R^2, lower bias of linear regressed line between observed versus predicted values from the 1:1 line and lower coefficient of variance (CV) was selected as the best model to estimate leaf area. *a* and *b* (as intercept and slope values of linear regression between observed versus predicted values of leaf area) were compared with zero and 1. a closer *a* to zero and closer *b* to 1 indicate better estimates of models. The final model was selected based on all statistical indices mentioned above. Equations were used in two formats: separately for each cultivar and pooled data to find a model to predict LA accurately for plants of all cultivars.

MODELS VALIDATION

In order to validate the selected models for estimated LA, about 100 leaves from each cultivar were randomly at the R1 to R6 growth stages collected and AA, L and W were determined by the previously described procedures. These values were used as independent data to validate the models (were not used in model fitting). Also, 20 plants selected and their actual leaf area and related dry weight were determined to plot against each other. LA was determined by the digital area meter. This was done just for fitting separate model on LA against leaf weight (Models: 7, 14, 21 and 28, **Table 3**). The slope and intercept of the model were tested to see if they were significantly different from the slope and intercept of the 1:1 correspondence line. Regression analyses were conducted using the SAS software (SAS Institute 1992).

RESULTS AND DISCUSSION

In different crops, different mathematical models have been used for indirect estimation of LA. The results were in agreement with some of the previous studies mentioned above on non-destructive models development for predicting leaf area using simple linear leaf measurements. Different functions with different independent variables including L, ΣL, W, ΣW, LW, ΣLW and DM were formulated to estimate LA. For this purpose, seven equations were selected for each cultivar as the best models to estimated related LA to leaflet, trifoliate and total leaf area. This was done by pooled data using the previously described procedures (**Table 3**). The relationship between L, ΣL, W, ΣW, LW, ΣLW and DM and related measured LA are presented in **Fig. 2**. These results indicated that a general equation can be advised to estimate leaflet, trifoliate and total leaf area of each cultivar (**Table 3**). Based on Statistical indexes previously described, we concluded that the models with single measurement on L did not perform well to estimate LA. In contrast, the LW-based models showed the best LA estimation. Although LW-based equations had higher R^2, lower RMSE and lower CV than other equations, but these models rely on two variables measurement (L and W per leaf) which is laborious and time consuming. So the W-based equations are preferred, due to simplicity and convenience. To validate the selected models, comparisons were made between measured versus predicted LA by using independent data on 'Dpx', 'Sahar' and 'Williams' cultivars which collected during 2009-2010 (equations 22 to 28, **Table 4**). Estimated values by the models were strongly consistent with the measured values of single leaves (**Table 4**). The regression line between measured versus predicted values did not show significant bias from the 1:1 line (*a* and *b* coefficients, **Table 4**). Models validation results demonstrate that soybean leaflet, trifoliate and total leaf area could be measured quickly, accurately, and non-destructively by using the developed models.

CONCLUDING REMARKS

In this study, rapid and simple models were developed to predict the leaf area of soybean cultivars. These models were selected according to simplicity, producing results with the same level of accuracy as other more complex

Table 3 Selected mathematical models for leaf area estimation of soybean cultivars without (regular values) and with (bold values) pooled data.

Cultivars	Cl.	E.n.	Var	Reg.M	Con.			R^2	RMSE	CV
					a	b	c			
Dpx	LL	(1)	W	$LA = a \times W^b$	1.59	1.82	-	0.97	5.2	11.5
	LL	(2)	LW	$LA = a + b \times LW$	- 1.33	0.68	-	0.99	2.9	6.40
	TL	(3)	W	$LA = a \times W^b$	3.60	1.93	-	0.96	17.3	13.5
	TL	(4)	LW	$LA = a + b \times LW$	- 8.11	2.01	-	0.98	11.3	8.80
	TA	(5)	ΣW	$LA = a + b \times \Sigma W$	- 25.07	21.9	-	0.97	128	7.40
	TA	(6)	ΣLW	$LA = a + b \times \Sigma LW$	- 17.01	1.91	-	0.99	71.5	4.10
	TA	(7)	DM	$LA = a + b \times DM + c \times DM^2$	- 22.14	272	- 0.7	0.95	898	15.9
Sahar	LL	(8)	W	$LA = a \times W^b$	1.66	1.83	-	0.97	3.4	11.4
	LL	(9)	LW	$LA = a + b \times LW$	0.08	0.67	-	0.99	1.9	6.40
	TL	(10)	W	$LA = a \times W^b$	2.04	3.10	-	0.97	10.6	13.1
	TL	(11)	LW	$LA = a + b \times LW$	- 7.76	1.99	-	0.98	6.9	8.60
	TA	(12)	ΣW	$LA = a + b \times \Sigma W$	- 125.7	19.19	-	0.97	93.4	7.50
	TA	(13)	ΣLW	$LA = a + b \times \Sigma LW$	5.65	1.79	-	0.99	63.7	5.10
	TA	(14)	DM	$LA = a + b \times DM + c \times DM^2$	- 150.3	318	- 1.5	0.96	817	16.7
Williams	LL	(15)	L	$LA = a \times L^b$	0.31	2.14	-	0.95	5.5	14.8
	LL	(16)	LW	$LA = a + b \times LW$	- 0.09	0.69	-	0.99	2.2	5.90
	TL	(17)	W	$LA = a + b \times W + c \times W^2$	16.91	7.71	3.7	0.96	12.8	12.8
	TL	(18)	LW	$LA = a + b \times LW$	- 8.95	2.01	-	0.98	9.5	9.50
	TA	(19)	ΣL	$LA = a + b \times \Sigma L$	4.41	1.19	-	0.92	175	14.2
	TA	(20)	ΣLW	$LA = a + b \times \Sigma LW$	- 5.95	1.83	-	0.99	64.8	5.20
	TA	(21)	DM	$LA = a + b \times DM + c \times DM^2$	- 441.2	324	- 1.5	0.96	707	12.9
Pool-D	LL	(22)	W	$LA = a \times W^b$	1.62	1.81	-	0.97	4.5	11.8
	LL	(23)	LW	$LA = a + b \times LW$	- 0.22	0.68	-	0.99	2.5	6.50
	TL	(24)	W	$LA = a \times W^b$	3.43	1.95	-	0.96	14.9	14.2
	TL	(25)	LW	$LA = a + b \times LW$	- 8.07	2.01	-	0.98	9.7	9.20
	TA	(26)	ΣW	$LA = a + b \times \Sigma W + c \times \Sigma W^2$	125.1	14.4	0.04	0.92	170	12.4
	TA	(27)	ΣLW	$LA = a + b \times \Sigma LW$	- 4.96	1.85	-	0.99	67.9	4.90
	TA	(28)	DM	$LA = a + b \times DM + c \times DM^2$	- 322.4	321	- 1.6	0.95	863	15.6

a: intercept, b: slope, c: constant, Cl. class, Con: constants, CV: coefficient of variance %, DM: green leaf dry matter, E.n.: equation number, L: length, ΣL: summed lengths, LL: leaflet, LA: leaf area, LW: length and width product, ΣLW: summed length and width product, Pool-D: pooled data R^2: determination coefficient, Reg-M: Regression model, RMSE: root mean square error, , TA: total leaf area, TL: trifoliate, Var: variable, W: width, ΣW: summed widths

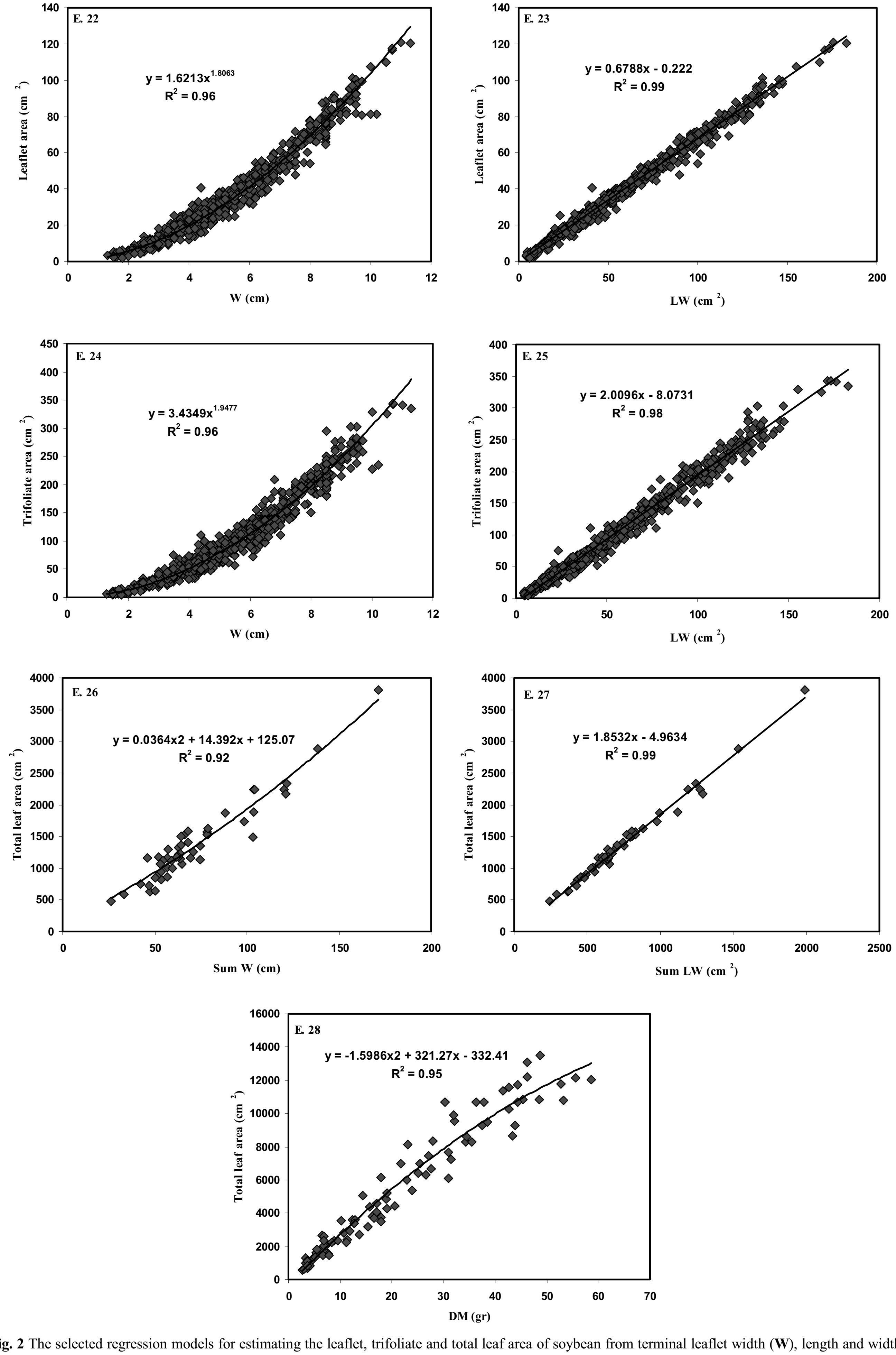

Fig. 2 The selected regression models for estimating the leaflet, trifoliate and total leaf area of soybean from terminal leaflet width (**W**), length and width product (**LW**), summed widths, summed length and width product and leaf dry matter (**DM**) by pooled data. E: equation number (**Table 3**).

Table 4 Validation the selected models for estimation leaflet, trifoliate and total leaf area for 'Dpx', 'Sahar' and 'Williams' cultivars by pooled data models.

Cultivars	E.n.	22	23	24	25	26	27	28
Sahar	R^2	0.96	0.98	0.97	0.97	0.94	0.99	0.94
	RMSE	3.32	2.34	9.08	8.41	147.47	64.67	825.4
	CV	11.76	8.09	11.96	10.74	10.16	4.93	20.07
	a	0.61 ns	0.56 ns	1.72 ns	-0.17 ns	189.99 ns	18.84 ns	- 40.31 ns
	b	0.95 ns	0.98 ns	0.96 ns	1.01 ns	0.96 ns	0.99 ns	0.99 ns
Dpx	R^2	0.98	0.99	0.97	0.98	0.99	0.99	0.90
	RMSE	3.93	2.31	15.74	11.41	94.14	40.97	1302
	CV	8.85	5.09	12.69	9.01	5.79	2.33	22.49
	a	- 1.23 ns	0.92 ns	- 3.89 ns	3.14 ns	- 86.98 ns	43.02 ns	- 304.5 ns
	b	1.02 ns	0.99 ns	1.02 ns	0.99 ns	0.96 ns	0.96 ns	1.01 ns
Williams	R^2	0.96	0.99	0.96	0.98	0.78	0.99	0.72
	RMSE	4.24	2.37	11.56	9.23	137.56	51.41	1514.4
	CV	10.90	6.34	10.81	8.95	10.07	3.88	20.85
	a	3.69 ns	0.28 ns	12.46 ns	3.64 ns	579.6 ns	47.94 ns	533.9 ns
	b	0.93 ns	0.98 ns	0.94 ns	0.99 ns	0.54 ns	0.96 ns	0.87 ns

a: intercept, b: slope, CV: coefficient of variance, E.n. equation number, R^2: determination coefficient
RMSE: root mean square error

estimation models or expensive equipments. The results demonstrated that soybean leaflet, trifoliate and total leaf area could be predicted using simple linear measurements. Dimensions of the leaves can be easily measured in the field, greenhouse and pot experiments. Use of these equations would enable researchers to make non-destructive or repeated measurements on the same leaves. With these developed models, researchers can estimate the LA of soybean cultivars accurately and in large quantities to use in physiological and quantitative studies. In general, our findings revealed that pooled data-based models (without respect to cultivar) are reliable for estimating the total leaf area, leaflet and trifoliate area, and researchers can use these models readily and without any inconvenience to save time and costs.

ACKNOWLEDGEMENTS

We acknowledge Mr E. Golghin for his kind help during the experiment.

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