Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 6 (7): 1037-1043, 2007
ISSN 1682-3974

Leaf Area Distribution Pattern and Non-Destructive Estimation Methods of Leaf Area for *Stevia rebaudiana* (Bert.) Bertoni

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Abstract: Leaf area is a valuable index for evaluating growth and development of sweet herb Stevia [*Stevia rebaudiana* (Bert.) Bertoni]. A simple methodology was developed during 2006 to estimate the leaf area through Leaf Area Distribution Pattern (LADP) and regression equations. Plant height, leaf height as well as the length and breadth of all the measurable leaves were measured and their area was measured through Area meter (AM 300) for a six month old crop of Stevia. A leaf area coefficient of 0.548 was found to fit for the linear equation without intercept. LADP was prepared with relative leaf height and relative leaf area. Based on the adjusted second order polynomial equation of LADP, the relative leaf height of plants representing the mean leaf area was ascertained and a regression equation was obtained to calculate the total leaf area of the plant. The results were validated with 3, 4 and 5 months old crops as well as with another accession. Different combinations of prediction equations were obtained from length and breadth of all leaves and a simplest equation i.e, linear equation was used to predict the leaf area. A non-destructive methodology for estimating leaf area of Stevia based on linear measurement was developed in this study.

Key words: *Stevia rebaudiana*, leaf area distribution pattern, relative leaf height, relative leaf area, prediction equations, regression equation

INTRODUCTION

Many countries have shown interest in cultivation of Stevia (*Stevia rebaudiana*), a sweet herb and research activities have been initiated. Incorporation of this species in agricultural production systems, however, depends upon a thorough knowledge of the plant and its agronomic potential (Ramesh *et al*., 2006). Leaf is an important organ and is associated with photosynthesis and evapotranspiration; therefore leaf area measurements are required in most of the physiological and agronomic studies. Direct methods of determining leaf area through tracing, shadow graphing etc to measure the leaf area of leaves attached to shoots is time consuming and tedious; also, in some experiments time is insufficient to make such measurements (Manivel and Weaver, 1974). The nondestructive methods reduce some of the experimental variability associated to destructive sampling procedures (NeSmith, 1992).

The use of regression equations to estimate leaf area is a nondestructive, simple, quick, accurate, reliable and not expensive method. The usual procedure of this method involves measuring lengths, breadths and areas 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). The non-destructive methods based on linear measurements are quicker and easier to be executed and present good precision for the study of plant growth in several crops (Robbins and Pharr, 1987). Therefore, a rapid and nondestructive method for measuring leaf area is required by the crop management specialists. Mathematical relationships between length, breadth and area of Stevia leaf can serve as a basis for direct leaf area estimation. Although, several prediction models are available to estimate leaf area for numerous crops, no information is available for *S. rebaudiana*.

The objectives were, to develop prediction equation to estimate *S. rebaudiana* leaf area, to determine whether prediction equations derived from independent variables involving measurements of both length and breadth were superior to those involving only measurements of length or breadth and to study the leaf area distribution pattern to estimate the leaf area and to develop a widely valid method to directly estimate, from length and breadth of a particular leaf, total leaf area of *S. rebaudiana*.

MATERIALS AND METHODS

Two months old Stevia seedlings were planted in a well prepared field in the experimental farm of Institute of

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Himalayan Bioresource Technology (CSIR), Palampur, India at a spacing of 45×45 cm during 2006. The site is located at an altitude of 1300 m above mean sea level (30°N and 76°E) and has a mean annual temperature of 18°C. Rainy season accounts for about 65% of the total annual rainfall exceeding 2500 mm and is consequently associated with low sun shine hours. Standard crop management practices were followed and need based crop protection measures was resorted.

Four months after transplanting, ten representative plants were selected at random and all the measurable leaves were detached carefully. These were taken to the laboratory and length (L) and the breadth (W) and area of all leaves were measured with an Area Meter (AM 300), ADC Bio-scientific Ltd, UK. Since Stevia had alternate leaf arrangement pattern, there was negligible variation between two leaves present opposite to each other. 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 external factors. This work examined the relationship between area per leaf and length and breadth dimensions in an attempt to identify appropriate functions for use in models estimating leaf area of Stevia.

The sampled leaf range for model development and its validation are shown in the Table 1.

Model development: To obtain, $Y = b\chi$ (Y is leaf area and χ is the leaf dimension parameter) the regression line was assumed to pass through the origin and the mean leaf coefficient/leaf area factor b was obtained from the following formula: Mean leaf coefficient/leaf area

$$\text{Factor} = \text{LAi}/ (\text{Li})(\text{Wi})$$

Where:

LAi = Actual leaf area of the ith leaf,
Li = Length of i th leaf,
Wi = Breadth of the i th leaf. Other prediction equations were also developed through an electronic (Microsoft Excel) work sheet.

Model validation: Two data sets were used to validate our model. The first data set was collected from a close planting of the crop similar to nursery (3, 4 and 5 months old). The second data set was collected from a field experiment of another accession with same spacing used for model development. The accuracy of the model predictions were estimated by regressing predicted data with observed data (coefficient of determination; R^2). The Root Mean Square Error (RMSE)/Standard Error of Treatment Means (SEm) of the mean weighted difference between observed and predicted values were also used.

$$\text{SEm} = \sqrt{\frac{\sum_{i=1}^{n}(\text{Sim} - \text{Obs})^2}{n}}$$

Where:

Sim = The predicted value,
Obs = The observed value,

A smaller value of RMSE indicated less deviation of the predicted values from the observed values (McMaster *et al*., 1992). Besides, Coefficient of variation was also used to validate the models. Coefficient of variation was calculated from the following equation:

$$\text{CV} = \text{SEm} \times 100/\chi$$

Where:

χ = The mean of observed values.

Leaf area distribution pattern: In addition, plant height and insertion height of the petiole from each leaf was also obtained to calculate the Relative Leaf Height (RLH) by the following equation:

$$\text{RLH} = \text{LH}/\text{PH}$$

where, LH is the distance between the soil surface and the node corresponding to the leaf and pH the plant height (cm). In the same day all the leaves were collected to

Table 1: Sample range for model development and validation

	Age of the plants (Months)				
	Accession-I				
Parameters	6*	5	4	3	Accession-II
Plant height (cm)	49-62	47-70.1	21.2-30	19-29	36-60.2
Total No. of leaves	28-36	26-34	18-22	12-20	28-52
Leaf length (cm)	3.07-9.65	4.04-10.39	1.71-7.04	1.3-4.88	1.6-8.05
Leaf breadth (cm)	1.24-3.86	1.5-3.23	1.14-3.58	0.86-2.24	1.12-5.33
Mean leaf area (cm^2)	6.94-13.08	7.73-11.45	4.64-6.89	2.66-4.31	6.61-15.57
No of leaves sampled	300	136	100	80	360

*: Used for model development

represent full spectrum of measurable leaf sizes. Length was measured from lamina tip to the point of intersection of the lamina and petiole and breadth was measured from tip to tip between the widest lamina lobes. The Relative Leaf Area (RLA) was calculated by dividing the area of each leaf by the mean leaf area of the plant for each RLH as follows.

$$RLA = LA/LA_M,$$

Where:

RLA = The relative leaf area

LA = The leaf area of the given leaf (cm^2) and

LA_M = The mean leaf area of the plant (cm^2 $leaf^{-1}$) derived by the sum of the LA of all leaves divided by the number of leaves in each plant.

The RLH was plotted against RLA in order to determine the leaf whose leaf area represents the mean leaf area of the plant. These leaves were selected and a regression equation was obtained to estimate the total leaf area of a single plant.

RESULTS

Developing prediction equation: Our first objective was to determine the most precise model to predict Stevia leaf area. An important consideration in developing prediction equation by regression analysis is the choice of the independent variable to be used. Therefore, different prediction equations involving different independent variables viz., Maximum leaf length (L, L^2, $L^{0.5}$) for length dimension, Maximum leaf breadth (W, W2, $W0^{0.5}$) for breadth dimension and combination of length breadth $L+W$, $(L+W)^2$, L^2+W^2, $(L^2+W^2)^{0.5}$, $LW^{0.5}$, $WL^{0.5}$, LW and $(LW)^{0.5}$ were formulated for estimating leaf area by using the following viz. $Y = a+b\chi$; $a+b\chi+c\chi^2$; $a+b \log_e \chi$; $a\chi^b$ and $ae^{b\chi}$.

Relation between leaf length (χ) and leaf area (Y): Leaf area prediction equations considering leaf length as independent variable are presented.

Prediction equations:

$$Y = 1.4864+0.1777\chi$$

Where, $\chi = L^2$ ($R^2 = 0.82$)

$$Y = 25.283-25.043\chi+7.3078\chi^2$$

Where, $\chi = L^{0.5}$ ($R^2 = 0.82$)

$$Y = 0.4849\chi^{1.5626}$$

Where, $\chi = L$ ($R^2 = 0.81$)

$$Y = 1.5802e^{0.2609\chi}$$

Where, $\chi = L$ ($R^2 = 0.82$)

(* Only equations with highest R^2 among the selected variables is presented).

Relation between leaf breadth (χ) and leaf area (Y): Leaf area prediction equations considering leaf breadth as independent variable for leaf area estimation are presented.

Prediction equations:

$$Y = 1.1588+1.2119\chi$$

Where, $\chi = W^2$ ($R^2 = 0.83$)

$$Y = 26.425-42.253\chi + 19.621\chi^2$$

Where, $\chi = W^{0.5}$ ($R^2 = 0.83$)

$$Y = -3.3337 + 14.043 \log_e \chi$$

Where, $\chi = W$ ($R^2 = 0.75$)

$$Y = 1.9276\chi^{1.6507}$$

Where, $\chi = W$ ($R^2 = 0.81$)

$$Y = 1.4623e^{0.6967\chi}$$

Where, $\chi = W$ ($R^2 = 0.82$)

(* Only equations with highest R^2 among the selected variables is presented).

Relation between leaf length and breadth (χ) and leaf area (Y): Leaf area prediction equations when leaf length and breadth considered together for leaf area estimation are presented. The Coefficient of determination ranged from 0.83-0.91 for L+W, 0.83-0.91 for $(L+W)^2$, 0.79-0.87 for L^2+W^2, 0.79-0.87 for $(L^2+W^2)^{0.5}$, 0.85-0.92 for $LW^{0.5}$, 0.86-0.94 for $WL^{0.5}$, 0.86-0.94 for $(LW)^{0.5}$ and 0.88-0.95 for LW.

Prediction equations:

$$y = 0.2667+0.528\chi$$

Where, $\chi = LW$ ($R^2 = 0.95$)

$$y = 0.9989 + 0.4346\chi + 0.0025\chi^2$$

Where, $\chi = LW\ (R^2 = 0.95)$

$$y = -12.51 + 7.9506 \log_e \chi$$

Where, $\chi = LW\ (R^2 = 0.88)$

$$y = 0.6513\chi^{0.9368}$$

Where, $\chi = LW\ (R^2 = 0.96)$

$$y = 1.2185e^{0.481\chi}$$

where, $\chi = (LW)^{1/2}\ (R^2 = 0.94)$

(* Only equations with highest R^2 among the variables are presented).

For each group, the fitted models were ranked according to their Coefficient of determination (R^2). The relative merits of defining LA with models were tested using R^2 value of each model. The model with the highest R^2 most frequently across all groups was regarded as the best model. From the above, based on R^2 value it was found that the use of both length and breadth of the leaves best represented the actual leaf area. LW product and actual leaf area for different models is shown in (Fig. 1).

Leaf area factor: When $y = b\chi$, the regression line was assumed to pass through the origin and the mean leaf coefficient/leaf area factor b was found 0.55 (Fig. 2). This was sufficiently enough ($R^2 = 0.95$) for other data sets with higher density planting of the same accession. The

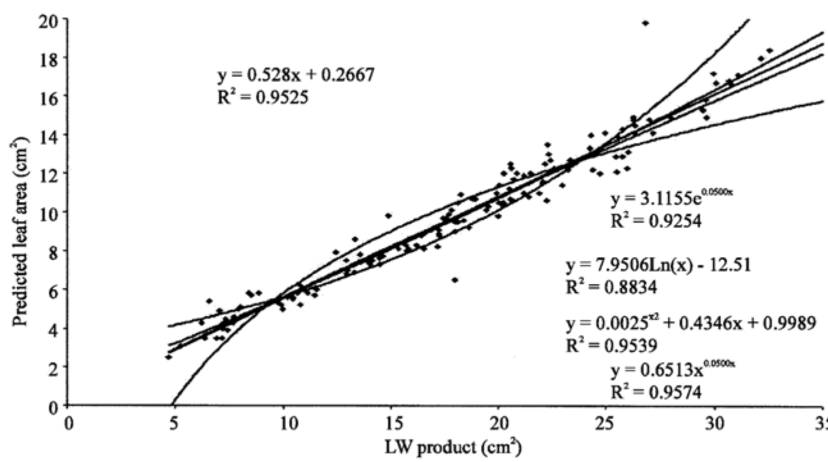

Fig. 1: LW product and actual leaf area for different models

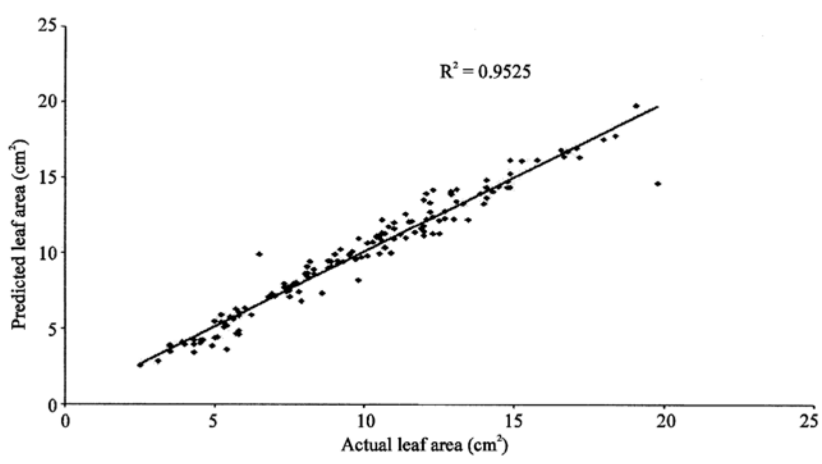

Fig. 2: Comparison observed and predicted leaf area (Leaf area factor method)

standard deviation for the coefficient was 0.053. The measurements more or less fall on to the same regression line for y = bx (R^2 = 0.98, 0.97 and 0.97 for 3,4 and 5 months, respectively)) although there was more scatter in the accession 2 with the same density planting (R^2 = 0.91) (Fig. 3).

Leaf Area Distribution Pattern (LADP): The RLA increases from the bottom of the plant and reaches a maximum value at RLH of about 0.5, when it decreases again until it reaches the plant apex. A high regression coefficient (was R^2 = 0.96) observed between the RLA and RLH, which adjusted to a second order

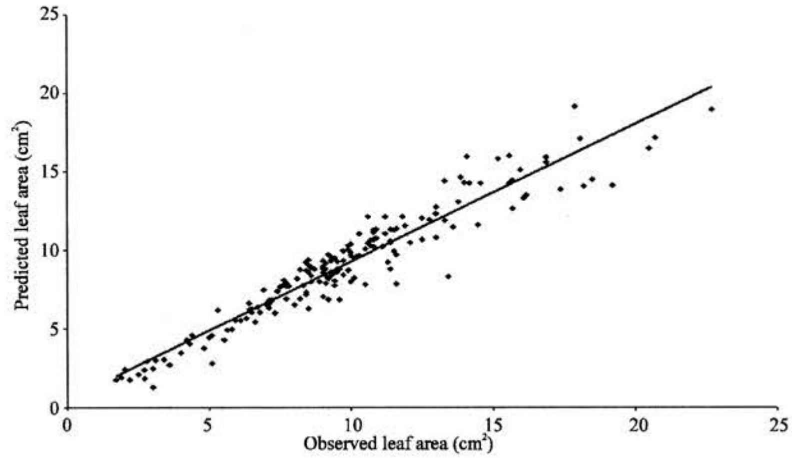

Fig. 3: Relationship between observed and predicted leaf area for accession 2 of Stevia

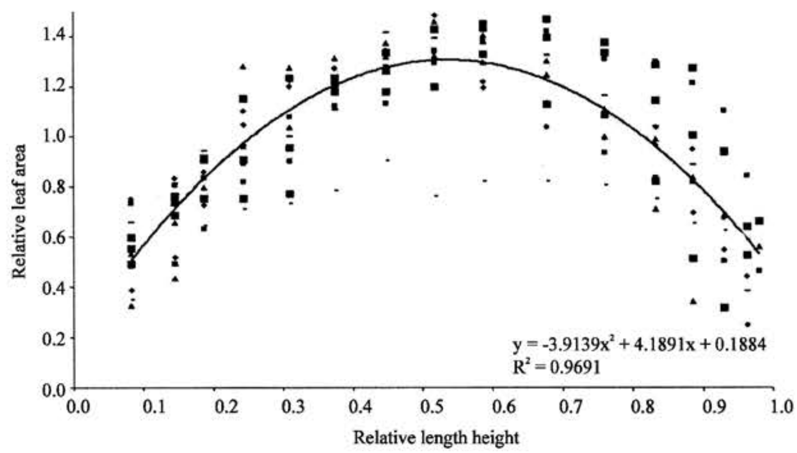

Fig. 4: Leaf area distribution for 6 months old stevia crop, curve represents mean of the values

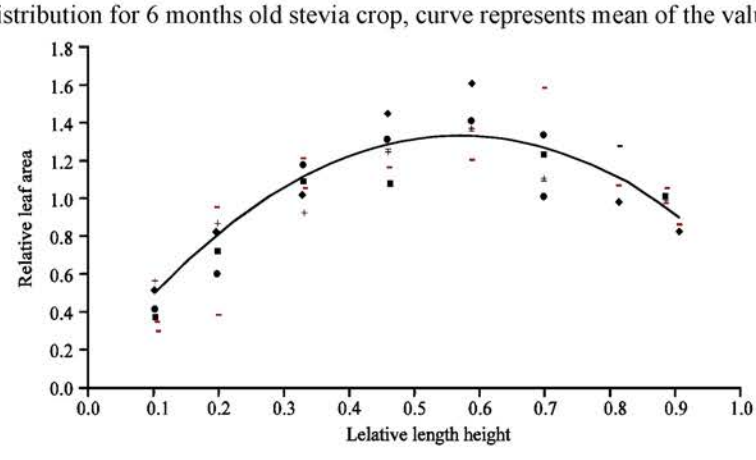

Fig. 5: Leaf area distribution for 3 months old Stevia crop, curve represents mean of the values

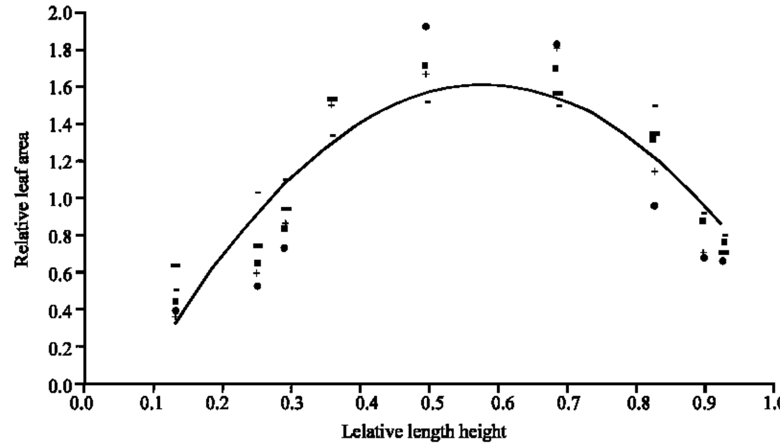

Fig. 6: Leaf area distribution for 4 months old Stevia crop, curve represents mean of the values

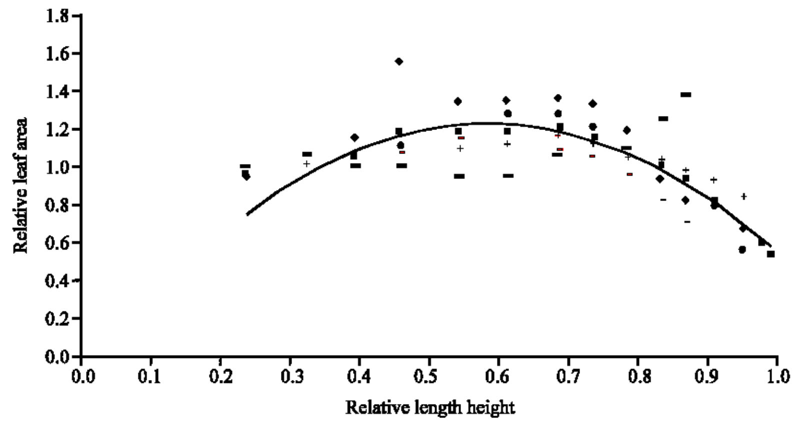

Fig. 7: Leaf area distribution for 5 months old Stevia crop, curve represents mean of the values

Table 2: Statistical significance of linear equation

S.No	Age of the crop (Months)	*R^2	RMSE	CV (%)
1	3	0.98	0.29	8.89
2	4	0.97	0.69	11.96
3	5	0.97	0.47	0.05
4	Accession 2	0.91	1.42	14.63

*: Based on linear regression; R^2: Coefficient of determination, RMSE: Root Mean Square Error; CV: Coefficient of Variation

polynomial equation for the crop (Fig. 4). Similar LADP curves were obtained for 3, 4, 5 months crop as well as the accession (Fig. 5-7).

Model validation: For the sake of simplicity, the linear equation derived above involving both leaf length and breadth product as independent variable was selected (y = 0.528x + 0.2667) and was validated for 3,4,5 months old crop and another accession (Table 2).

DISCUSSION

The economic importance of *S. rebaudiana*: solely depends on the content of stevioside and rebaudioside A (Ramesh *et al*., 2007) in the leaves. Results of single dimension approach did not provide satisfactory results and so two dimension approach was adopted. The most appropriate model included both the length and breadth dimensions. Montgomery (1911) first suggested that leaf area of a plant can be calculated from linear measurement of leaves using a general relationship A = b×I×W where b is a coefficient, A is area of the leaf, I is length of the leaf and W is breadth of the leaf. The original routine in the calculation of coefficient model uses a factor which has to be determined from destructive experimental sampling similar to the procedure followed by McKee (1964). The coefficient 0.548 was sufficiently enough (R^2 = 0.97) for other data sets with higher density planting of the same accession.

The models based on single variable measurement offer the advantages of more efficient data collection, less complex calculations (NeSmith, 1992) and require less time for leaf measurement (Robbins and Pharr, 1987). Nonetheless, they were less satisfactory for predicting leaf area as evident from the estimated coefficient of determination r^2 in different prediction equations. In accordance with the suggestions of Schneiter and Miller

(1981), that measurement of both breadth and length can be more precise than estimates based on one dimension for leaf area estimation in Sunflower. In the present study, the combination of length and breadth showed higher precision than as individual variables either L or W. On the basis of this we may select LW as independent variable for more reliability excepting for $y = ae^{bx}$ where $(LW)^{0.5}$ may be used.

The coefficient of regression was reported to be a good measure of predictive ability of a model (Wiersma and Bailey, 1975). All of the regressions were significant and all of the coefficients of determination exceeded 0.88. Similar prediction equations for leaf area measurement of several crops have been worked out for soybean (Wiersma and Bailey, 1975), frenchbean (Rai *et al*., 1988), sunflower (Chanda and Singh, 1997) and ramie (Sarkar and Maitra, 2001) etc.

The coefficient of determination, Root Mean Square Error (RMSE) and Coefficient of Variation showed the close predictability. The hypothesis that a common regression equation could be used to predict the leaf area of leaf at different stages was also verified for the said model. This suggested that either leaf area factor or a linear model can well be used to predict Stevia leaf area.

Leaf area distribution pattern: The LADP was adjusted to a second order polynomial equation. From the adjusted equations, it was possible to calculate the relative height of the leaf that represented the mean leaf area of the plant. Blanco and Folegatti (2003) developed this pattern for cucumber and tomato plants. The leaves that represented mean leaf area of the plant were that corresponding to a relative leaf height of 0.25 and 0.85 for this crop. The total leaf area can be calculated from the leaf area of the said representative leaves by multiplying the computed leaf area with the total number of leaves present in the plant at any given point of time.

The leaf area coefficient of Stevia is approximately constant despite variations in density of planting and genotype too. Further, the exact values of regression constants a and b in prediction equations are not unique parameters. Thus, these equations are a balance between accuracy and simplicity. Either the leaf area factor 0.548 may be directly used to estimate single leaf area or the linear regression equation $y = 0.528 + 0.267\ \chi$ may be used with more predictability by utilizing both length and breadth of the leaves. Neither length nor breadth appeared better than the other as a basis of estimating the leaf area. Both length and breadth measurements were needed to attain precision in leaf area calculation. The leaves at the Relative leaf height of 0.25 and/or 0.85 (Fig. 3) will suggest the mean leaf area of the plant so that the total leaf area of the plant be estimated using the said models. The applicability of the suggested prediction equations to other environmental and management conditions is not known. The leaf area predicted here based on linear dimensions agreed well with data from the crop at different ages. Since no models were previously applicable for prediction of leaf area, this work could be a valuable contribution towards Stevia leaf area estimation.

ACKNOWLEDGMENTS

The authors thank The Director, IHBT for his constant encouragement for the work. The authors also thank Mr. Suresh Sood and Mr Kuldeep S Gill, Technical Assistants for the management of the experimental crop.

REFERENCES

Blanco, F.F. and M.V. Folegatti, 2003. A new method for estimating the leaf area index of cucumber and Tomato plants. Hortic. Brasileira, 21: 666-669.

Chanda, S.V. and Y.D. Singh, 1997. A rapid method for determining leaf area in sunflower. Acta Agron., 27: 70-75.

Manivel, L. and R.J. Weaver, 1974. Biometric correlations between leaf area and length measurements of Grenache grape leave. Hortic. Sci., 9: 27-28.

McKee, G.W., 1964. A coefficient for computing leaf area in hybrid corn. Agron. J., 56: 240-242.

McMaster, G.S., W.W. Withelm and J.A. Morgan, 1992. Simulating winter wheat shoot apex phenology. J. Agric. Sci. (Cambridge), 119: 1-12.

Montgomery, E.G., 1911. Correlation studies in corn. Nebraska Agric. Exp. Station Ann. Report, 24: 108-159.

NeSmith, D.S., 1992. Estimating summer squash leaf area nondestructively. Hortic. Sci., 27: 77-79.

Rai, A., P.B. Alipit and M.B. Toledo, 1988. Estimation of leaf area of French bean using linear measurement. Ind. J. Agric. Sci., 58: 727-729.

Ramesh, K., V. Singh and N.W. Megeji, 2006. Cultivation of Stevia (*Stevia rebaudiana*)-a comprehensive review. Adv. Agron., 87: 137-177.

Ramesh, K., V. Singh and P.S. Ahuja, 2007. Production potential of Stevia rebaudiana under intercropping systems. Arch. Agron. Soil Sci., 53: 443-458.

Robbins, N.S. and D.M. Pharr, 1987. Leaf area prediction models for cucumber from linear measurements. Hortic. Sci., 22: 1264-1266.

Sarkar, S. and D.N. Maitra, 2001. Prediction of leaf area in ramaie (*Boehmeira nivea* L.) gaud. By non-destructive method. Ind. J. Plant Physiol., 6: 403-405.

Schneiter, A.A. and J.F. Miller, 1981. Description of sunflower growth stages. Crop Sci., 21: 901-903.

Wiersma, J.V. and T.B. Bailey, 1975. Estimation of leaflet, trifoliate and total leaf areas of soybeans. Agron. J., 67: 26-30.