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A B S T R A C T

Leaf area estimation is an important biometrical observation one has to do for comparing plant growth in field and pot experiments. In this study, a leaf area estimation model was developed for ginger (*Zingiber officinale* Roscoe), using linear measurements of leaf length (L) and maximum width (W). Leaves from five ginger varieties (Varada, Rejatha, Mahima, Maran and Himachal) were used to develop the model in 2006–2007. The actual leaf area (LA) was measured with a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA) and taken as reference LA. The linear measurements were used to build linear ($LA = a + b \times L \times W$) and power models ($LA = \alpha \times (L \times W)^{\beta}$) for each variety, as the modeling among variety were not different from each other, data for all five varieties have been pooled and compared with earlier models by graphical procedures and statistical criteria such as Mean Square Error (MSE), Root Mean Square Error (RMSE) and Chi-square (χ^2). The selected model was validated during 2007–2008. The validation data set was used to produce a validation model for each variety by re-estimating the model parameters to develop the estimation model and the models were compared for consistency. The predicted LA (PLA) was compared with observed LA (OLA) by graphical procedures and lack of agreement was evaluated by calculating the relative bias, estimated by the mean of differences (d) and the standard deviation (SD) of the differences. Normality test was carried out by Spearman's rank correlation coefficient (r_s) and residuals were normally distributed. Finally, the proposed model for leaf area estimation of ginger is $LA = 0.0146 + 0.6621 \times L \times W$, $R^2 = 0.997$. This model can be reliably used for estimating leaf area of ginger non-destructively. The same equation can be extrapolated to all varieties and land races of ginger as it is vegetatively propagated crop with narrow genetic variability.

1. Introduction

Ginger (*Zingiber officinale* Roscoe), a monocotyledon belonging to family *Zingiberaceae* and in the natural order *Scitamineae*, is herbaceous perennial, usually grown as annually for its pungent rhizome. It is native of South East Asia and one of the earliest oriental spices known to Europe (Kandiannan et al., 1996; Parthasarathy et al., 2003; Ravindran and Nirmal Babu, 2005). Commercially more than 25 countries in the tropics and subtropics are producing it, however, India is the largest producer, and it is an export oriented crop (Singh and Tamil Selvan, 2003).

Leaves are important organs of the plant. Leaf area (LA) is a key variable for most agronomic and physiological studies involving plant growth, light interception, photosynthetic efficiency, evaporation, and responses to fertilizers and irrigation (Blanco and Folegatti, 2005). Therefore, LA strongly influences growth and productivity; estimating LA is a fundamental component of crop growth models (Lizaso et al., 2003). However, the measurement of the surface area of a large number of leaves is often costly, time consuming and destructive. The total LA of the plant can be obtained by either direct or indirect methods. The direct method consists of removing and measuring all leaves in plant. This method is destructive and requires expensive equipment. Indirect, non-destructive methods are user-friendly, less expensive, and can provide accurate LA estimates (Norman and Campbell, 1989) and help in *in situ* LA estimation. A modeling approach is rapid, reliable and involves linear relationships between LA and one or more dimension of the leaf (L – leaf length and W – maximum width) and it is an alternative for accurately measuring LA (Kandiannan et al., 2002; Williams and Martinson, 2003; Lu et al., 2004; Cho et al., 2007; Peksen, 2007; Antunes et al., 2008).

Although LA is an important parameter in growth studies, only a few workers from India, China and Australia reported for ginger (Gowda and Melanta, 2000; Xizhen et al., 2001; Ajithkumar et al., 2002; Ajithkumar and Jayachandran, 2003; Smith et al., 2004; Xizhen et al., 2005). Earlier methods, employing leaf dimension for estimating the area of ginger leaf was proposed $LA = -1.7362 + 0.7153 \times L \times W$ (Jayachandran and Sethumadhavan, 1979) with

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coefficients estimated using least-square linear regression analysis. Similar equations were also developed later LA = −0.7608 + 0.6695 × L × W (Ancy and Jayachandran, 1994) and LA = −9.358 + 0.8549 × L × W (Reddy and Reddy, 1995). It was also proposed an equation LA = k × L × W, k = 0.666 (Reddy and Reddy, 1995), a coefficient 'k' derived as the ratio of LA to the product of L and W. Nwachukwu and Ene (1987) compared different methods of estimating ginger LA from Nigeria and reported that no differences were noted between grid and punch method. Punch method was not recommended because of the time and labour required for its use and other calculation methods based on leaf length and width gave unrealistic results for ginger. The above equations have been selected based only on values obtained for the coefficient of determination (R^2) and without assessing their prediction accuracy by validation. They also have used limited number of samples for building equations and from only one variety Rio-de-Janeiro (Jayachandran and Sethumadhavan, 1979; Ancy and Jayachandran, 1994). Hence, no information is available on whether or not such models can be successfully used to other genotypes. Moreover, a graph paper method has been employed to find LA that might significantly underestimate the actual LA. Reddy and Reddy (1995) also used limited number of leaves from five genotypes and they have not validated their equation. In these studies, the adequacy of the model assumptions for estimating LA has not been carefully examined. A simple and effective method for detecting model deficiencies in regression analysis is the examination of residual (Bland and Altman, 1986; Rangaswamy, 1995). Therefore, it is necessary to have a simple and validated accurate model for LA estimation of ginger. In this study, we aimed to evaluate the current models (Jayachandran and Sethumadhavan, 1979; Ancy and Jayachandran, 1994; Reddy and Reddy, 1995), as well as to propose a reliable and accurate model using measurements of L and W for estimating the LA of ginger by non-destructive method.

2. Materials and methods

2.1. Data collection

Ginger plants were grown at Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi, Calicut District, Kerala State, India (geographical coordinates 11°34′N, 75°48′E and 60 m MSL) during 2006–2007 and 2007–2008 crop seasons. The region is located in the Western Ghat area of India encompasses one of the world's richest biodiversity. The site experiences tropical humid climate with mean annual rainfall of 4460 mm received from southwest (June–September) and north east (October–November) monsoons with major share from southwest monsoon (75%). Five ginger varieties viz., Varada, Rejatha, Mahima, Maran and Himachal (Table 1) were used for the study. Leaves were sampled randomly at the active growth stage from different plants and from different levels of the shoot for each variety during 2006–2007. A total of 765 leaves, 153 leaves of each variety, were measured in order to develop the best fitting model for predicting the LA of ginger. Maximum leaf width (W) (at the widest point perpendicular to the midrib) and length (L) (from lamina tip to the point of petiole intersection along the midrib) were measured to the nearest millimeter. The actual LA measured with a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA) and taken as reference. Similarly, for the selection of better model and testing the validity, new leaf samples were collected during 2007–2008. The means, standard deviations, minimum and maximum values of the leaf length and width and LA for each ginger variety used for model estimation, selection and validation are shown in Table 2.

2.2. Model building and validation

The linear measurement of L and W were used to build the models, as these variables jointly explain the large part of total

Table 1
Ginger varieties used for leaf area model construction.

Variety		Fresh yield (t ha^{-1})	Dry recovery (%)	Oleo-resin (%)	Essential oil (%)	Crude fibre (%)
Varada	Selection from germplasam	22.6	20.7	6.7	1.8	4.5
Rejatha	Selection from germplasam	22.4	19.0	6.3	2.4	4.0
Mahima	Selection from germplasam	23.2	23.0	4.5	1.7	3.3
Maran	Popular land race	25.2	20.0	10.0	1.9	6.1
Himachal	Popular land race	7.3	22.1	5.3	0.5	3.8

Table 2
Mean ± standard deviations, minimum (Min) and maximum (Max) values for the leaf length (L), width (W) and leaf area (LA) used for model building, selection and testing.

Variety	n	L (cm)			W (cm)			LA (cm^2)		
		Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max
For model building(2006–2007)										
Varada	153	16.4 ± 5.9	1.8	26.0	1.8 ± 0.7	0.3	3.0	21.54 ± 12.58	0.41	50.83
Rejatha	153	16.0 ± 5.3	1.5	26.0	1.8 ± 0.5	0.4	3.0	20.78 ± 10.42	0.40	50.49
Mahima	153	17.7 ± 5.4	2.5	28.0	2.0 ± 0.5	0.4	3.0	24.16 ± 10.61	0.68	56.17
Maran	153	16.9 ± 5.4	2.0	29.0	1.9 ± 0.5	0.4	2.9	22.47 ± 10.11	0.56	44.84
Himachal	153	16.5 ± 6.3	1.5	28.0	1.9 ± 0.6	0.2	2.9	23.03 ± 12.33	0.20	50.99
Pooled	765	16.7 ± 5.7	1.5	29.0	1.8 ± 0.5	0.2	3.0	22.38 ± 11.29	0.20	56.17
For model selection (2007–2008)										
Random sample	362	17.5 ± 5.4	2.0	27.0	2.0 ± 0.5	0.4	3.1	24.19 ± 11.55	0.55	57.80
For model validation(2007–2008)										
Varada	107	16.5 ± 4.4	5.0	24.4	1.8 ± 0.4	1.0	2.5	20.93 ± 8. 28	3.35	37.92
Rejatha	130	17.8 ± 5.3	4.0	26.0	2.0 ± 0.5	0.8	3.0	24.39 ± 11.79	2.22	57.79
Mahima	125	17.9 ± 6.3	2.0	27.0	2.1 ± 0.5	0.4	3.1	26.79 ± 13.00	0.54	56.86
Maran	144	15.9 ± 5.1	2.2	28.0	1.8 ± 0.5	0.6	2.7	20.16 ± 9.76	0.90	39.02
Himachal	159	19.1 ± 5.8	3.0	28.0	2.3 ± 0.5	1.0	3.4	29.87 ± 13.22	2.19	60.73
Pooled	665	17.5 ± 5.5	2.0	28.0	2.0 ± 0.5	0.4	3.4	24.68 ± 12.07	0.54	60.73

variation of LA (Cho et al., 2007; Peksen, 2007; Antunes et al., 2008). The actual LA was measured with an instrument. Then, relationships were evaluated by fitting regression models. Linear ($LA = a + b \times L \times W$) and power relationships ($LA = \alpha(L \times W)^{\beta}$) were fitted. When power models were fitted, both dependent and independent variables were subjected to logarithmic transformation before analysis. Individual models for each variety have been built and tested for their equality as suggested by Gomez and Gomez (1984) and Rangaswamy (1995). These two new models were compared with three previous models for prediction of ginger LA.

Five models (Models I–V) were used for LA estimation (Table 4). Mean deviation of estimated area from observed area of the individual ginger leaf for Models I–V was compared by graphical procedures (Graybill, 2000; Antunes et al., 2008) and based on statistical criteria (Table 5) such as coefficient of multiple determination R^2, adjusted coefficient of multiple determination R^2_a, standard error, intercept, standard error of intercept, regression coefficient, standard error of regression coefficient, Mean Square Error (MSE), Root Mean Square Error (RMSE), Pearson's correlation coefficient (r) and Chi-square (χ^2).

In order to validate the produced model over pooled data from all genotypes, leaf samples of 107, 130, 125, 144, and 159 from ginger varieties Varada, Rejatha, Mahima, Maran and Himachal, respectively were used. Data used in the model validation were obtained from the new leaf samples collected during 2007–2008. To validate the model, the validation data set was used to produce a validation model by re-estimating the model parameters to develop the estimation model and the models were compared for consistency as reported by Peksen (2007). The predicted LA (PLA) was compared with observed LA (OLA) for five varieties of ginger by graphical procedures described by Bland and Altman (1986). Normality test was carried out by Spearman's rank correlation coefficient (r_s). All the data was analyzed using SPSS10.0.1 programme.

3. Results

Ginger plants produce leafy shoot (pseudo-stem) about 50 cm tall. The aerial pseudo-stem bear distichous leaves, usually 5–25 cm long and 1–3 cm wide. The samples drawn for this study from five ginger varieties with their summary statistics are given in Table 2. There was no validated model available for prediction of LA of ginger. The sample data has been used for building estimation and validation models. Models were built separately for five varieties and test for their homogeneity was carried out (Table 3). The data was normally distributed and F-test indicated that calculated F value was lower than table value indicating their homogeneity. Therefore, data for these varieties were pooled and single regression models were fitted to the combined data. The single linear ($LA = -0.0146 + 0.6621 \times L \times W$) and power ($LA = 0.6604(L \times W)^{1.0003}$) model was built from pooled data and compared with previous models (Table 4). The predictability of new models was better compared to earlier models. Because, while comparing the mean deviation of differences between predicted and observed LAs of models (Fig. 1), it was minimum (0.1059 cm^2) in Model I that is built from this study. Similarly, comparing the values of MSE, RMSE and Chi-square (χ^2) of models (Table 5), Model I has recorded the least value for these statistics that is commonly used to select the best model. Thus, Model I has been selected for the estimation of LA for ginger varieties.

R^2, R^2_a, and parameters estimates (Table 6) showing similarity for the estimation and validation models give some assurance

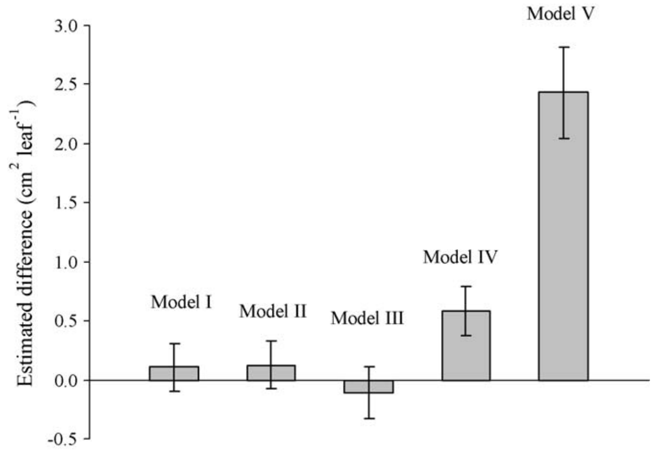

Fig. 1. Mean deviation of predicted area from observed area of the individual ginger leaf for Models I–V. Vertical bars denote means and spreads denote 95% confidence intervals of the difference.

Table 3
Comparisons for testing the significances of differences between slopes and intercepts of five (varieties) simple linear regressions.

Regressions	SS (LA)	SS (*LW*)	SP (LA × *LW*)	*n*	*b*	Error SS	Error d.f.
Varada	95,491.1	219,842.0	144,719.5	153	0.6582	223.3011	151
Rejatha	82,499.1	188,849.6	124,787.2	153	0.6607	44.0133	151
Mahima	100,188.1	228,590.5	151,300.4	153	0.6618	45.6752	151
Maran	92,842.3	211,054.6	139,937.0	153	0.6630	58.7675	151
Himachal	104,266.7	236,112.1	156,871.1	153	0.6643	42.2743	151
Pooled	–	–	–	–	–	414.031	755
Common	475,287.4	1,084,449.0	717,615.3	–	–	417.9391	–
Total	481,064.5	1,097,537.0	726,308.7	765		420.6323	763

Calculated F value for comparing regression coefficients, regression lines and intercepts are 1.7813, 1.5045 and 1.2163, respectively and critical F value for 4 and 755 (∞) d.f. and for $\alpha = 0.001$ is 2.37 and F value for 8 and 755 (∞) d.f. and for $\alpha = 0.001$ is 1.94.

Table 4
Regression models used for estimating ginger LA in this study.

Models	Equation	R^2	Source
Model I	$LA = -0.0146 + 0.6621LW$	0.9956	New model from this study
Model II	$LA = 0.6604(LW)^{1.0003}$	0.9987	New model from this study
Model III	$LA = 0.7153LW - 1.7362$	–	Jayachandran and Sethumadhavan (1979)
Model IV	$LA = 0.6695LW - 0.7608$	0.986	Ancy and Jayachandran (1994)
Model V	$LA = 0.8549LW - 9.358$	0.9506	Reddy and Reddy (1995)

Table 5
Statistics and parameter estimates from regression models for LA estimation of ginger ($n = 362$) for comparison of models.

Statistic or Parameters estimate	Model I	Model II	Model III	Model IV	Model V
Coefficient of multiple determination R^2	0.9704	0.9704	0.9704	0.9704	0.9704
Adjusted coefficient of multiple determination, R_a^2	0.9703	0.9703	0.9703	0.9703	0.9703
Standard error	1.9887	1.9887	1.9887	1.9887	1.9887
Intercept	−0.1955	−0.2046	1.4169	0.5515	7.1279
Standard error of intercept	0.2475	0.2476	0.2341	0.2413	0.1886
Regression coefficient	1.0125	1.0137	0.9372	1.0013	0.7841
Standard error of regression coefficient	0.0093	0.0093	0.0086	0.0092	0.0072
Mean Square Error (MSE)	3.9643	3.9728	4.5251	4.2731	19.6217
Root Mean Square Error (RMSE)	1.9910	1.9932	2.1272	2.0671	4.4296
Pearson's correlation coefficient (r)	0.9851	0.9851	0.9851	0.9851	0.9851
Chi-square (χ^2) (table value of χ^2 for d.f. 100 is 124.34 at $P = 0.05$)	45.4215	45.5281	77.2558	59.2884	−112.4276

about of the applicability of the models to data (Neter et al., 1996). The proposed LA estimation model is $\text{LA} = -0.0146 + 0.6621 \times L \times W$, $R^2 = 0.997$, where LA is leaf area (cm^2), L is leaf length (cm), and W is the maximum width of the leaf (cm). Regression analysis revealed that most of the determinable variations in leaf area values were explained by the measured leaf length and leaf width. The Pearson's correlation coefficient in the estimation model (0.997) was similar to that of validation models.

The scatter plot for PLA against OLA is presented in Fig. 2. The difference between predicted and observed LAs was calculated for each variety and for pooled data; these were plotted against mean of predicted and observed LAs. Correlation coefficient showed that PLA and OLA were strongly correlated. Correlation coefficient between PLA and OLA for Varada, Rejatha, Mahima, Maran and Himachal and pooled data were 0.997, 0.982, 0.983, 0.992, 0.977 and 0.984, $P < 0.001$, respectively. Correlation analysis alone was not sufficient to explain relationships between PLA and OLA. A plot of differences between PLA and OLA against their mean would be more informative (Bland and Altman, 1986) that is illustrated in Fig. 3. Lack of agreement between estimation of PLA and OLA was evaluated by calculating the relative bias, estimated by the mean of differences (d) and the standard deviation (SD) of the differences (Fig. 3). In Fig. 3, a centre solid line represents the mean of the differences. When the differences were distributed normally, 98% of the differences would lie between $d \pm 2\text{SD}$. In this study, a few plots were out of these lines while the rest of the plots were placed between lines. Spearman's correlation coefficient ($r_s = 0.1083$) and t-test ($t_{cal} = 2.805$, $t_{tab} = 3.29$, $P = 0.001$) revealed that the residuals were normally distributed.

4. Discussion

Leaf area is one of the important growth parameters and one must record it for effective monitoring of the growth and development of plant in the experiment. Lack of accurate model is a limitation for calculating LA. Non-destructive method of the

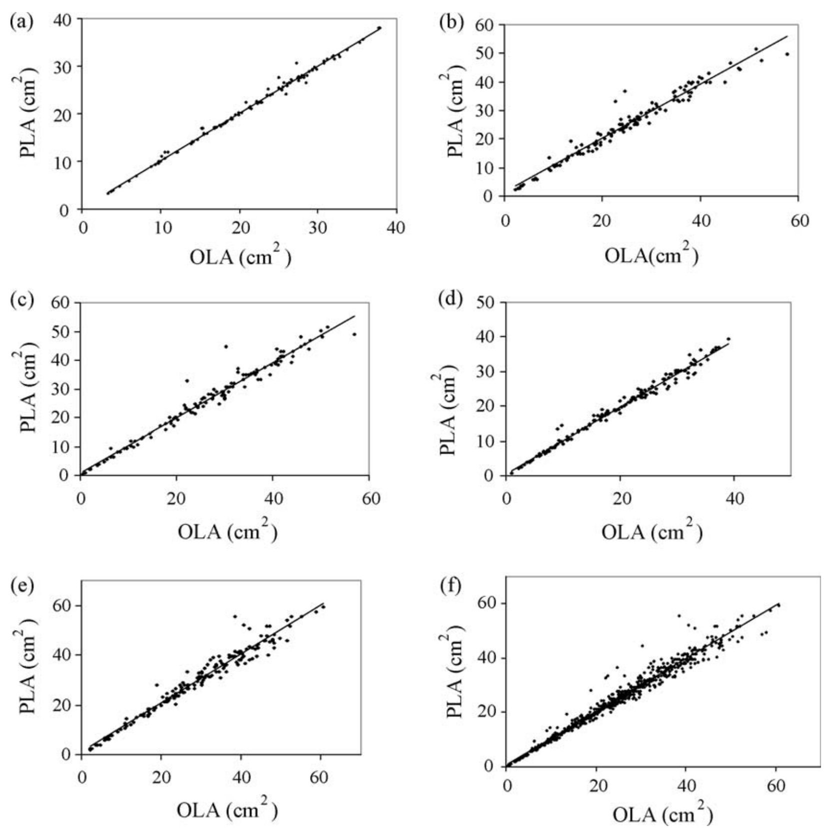

Fig. 2. Plot of predicted leaf area (PLA), estimated by model vs. the observed leaf area (OLA) for varieties (a) Varada, (b) Rejatha, (c) Mahima, (d) Maran, (e) Himachal and (f) Pooled data.

Table 6
Statistics and parameter estimates for comparing consistency of estimation and validation models for LA estimation of ginger.

Statistic or Parameters estimate	Estimation model	Validation models					
		Varada	Rejatha	Mahima	Himachal	Maran	Pooled
Coefficient of multiple determination R^2	0.996	0.995	0.966	0.967	0.985	0.955	0.969
Adjusted coefficient of multiple determination, R_a^2	0.996	0.995	0.966	0.967	0.985	0.955	0.969
Standard error	0.742	0.636	2.265	2.380	1.196	2.816	2.123
Intercept	−0.015	−0.079	−0.308	0.132	−0.289	0.216	0.125
Standard error of intercept	0.059	0.155	0.444	0.494	0.233	0.560	0.189
Regression coefficient	0.662	1.000	1.013	1.008	1.025	0.970	0.993
Standard error of regression coefficient	0.002	0.007	0.016	0.016	0.011	0.017	0.007
Mean Square Error (MSE)	0.549	0.415	2.721	2.645	1.505	3.602	2.896
Root Mean Square Error (RMSE)	0.741	0.644	1.649	1.626	1.226	1.898	1.702
Pearson's correlation coefficient (r)	0.998	0.999	0.981	0.983	0.993	0.977	0.984
Chi-square (χ^2) (table value of χ^2 for d.f. 100 is 124.34 at $P = 0.05$)	15.860	2.052	26.622	24.803	11.252	40.490	105.219

estimation of LA has several advantages without compromising on accuracy (Kandiannan et al., 2002; Williams and Martinson, 2003; Lu et al., 2004; Cho et al., 2007; Peksen, 2007; Antunes et al., 2008). An export oriented crop like ginger requires LA estimation often for comparing and evaluating germplasam and crop management studies (Smith et al., 2004; Xizhen et al., 2005; Lincy et al., 2008). Many researches have been carried out to estimate leaf area by measuring leaf dimensions. In general, the combination of leaf length (L) and maximum width (W) has been used as the parameters of leaf area models (Montero et al., 2000; Williams and Martinson, 2003; Lu et al., 2004; Cho et al., 2007; Peksen, 2007; Antunes et al., 2008). The building models and improvement is a continuous process. Various mathematical models for indirect estimation of leaf area of different plant species have been described (Campostrini and Yamanishi, 2001; Kandiannan et al., 2002; Bhatt and Chanda, 2003; Williams and Martinson, 2003; Demirsoy et al., 2004; Lu et al., 2004; Sousa et al., 2005; Gamper, 2005; Tsialtas and Maslaris, 2005; Serdar and Demirsoy, 2006; Cho

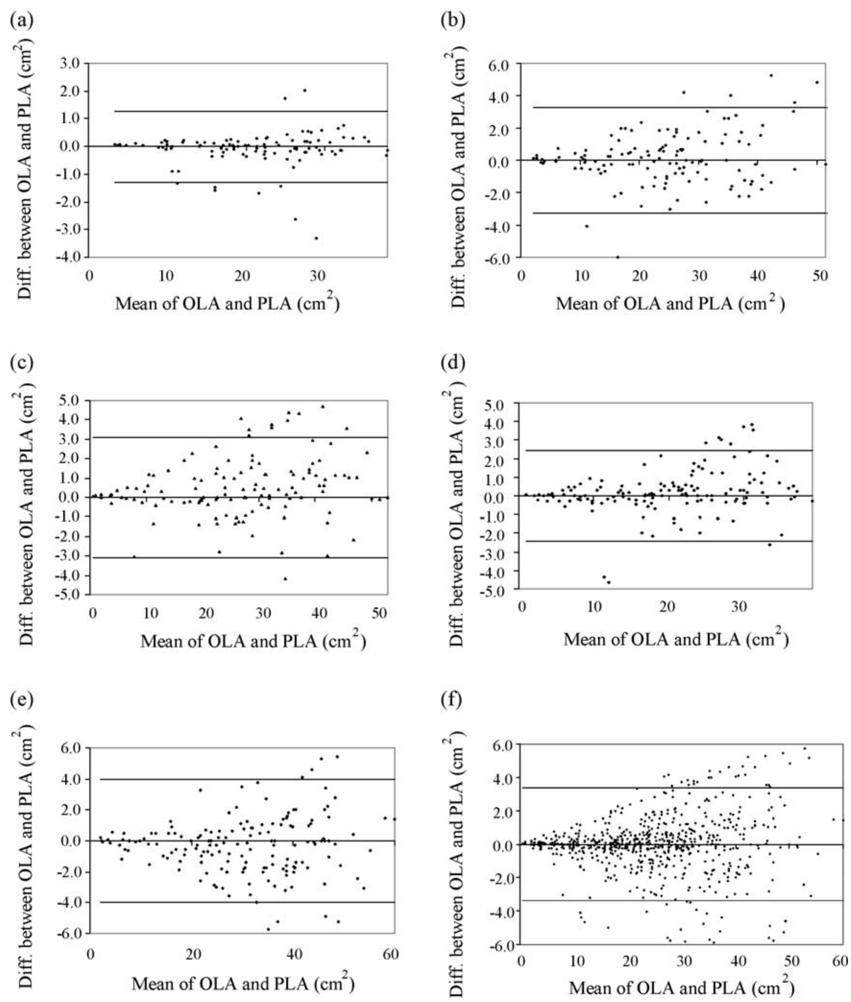

Fig. 3. The differences between observed leaf area (OLA) and predicted leaf area (PLA) by model for individual varieties and pooled data vs. the mean of observed leaf area (OLA) and predicted leaf area (PLA) for varieties (a) Varada, (b) Rejatha, (c) Mahima, (d) Maran, (e) Himachal and (f) Pooled data. The centre solid line is the mean differences. Solid lines on either side are limits of agreement, calculated as $d \pm 2SD$; where d = the mean of the differences and SD = the standard deviation of the differences. If the differences are normally distributed, 98% of the differences in a population would lie between the limits of agreement.

et al., 2007; Peksen, 2007; Antunes et al., 2008). Present study results were in agreement with some of the previous studies mentioned above on non-destructive model development for predicting leaf area using simple linear measurements. In the study, very close relationships were found between actual leaf area and predicted leaf area using the model. The validation of the model showed that ginger leaf area could be estimated quickly, accurately, and non-destructively by using the developed model. The same model can be extrapolated to any ginger variety, type or land race. Because of lack of seed set in ginger, the crop is always propagated vegetatively and its genetic variability is very narrow, distinctive ginger types at different production centres exists due to soil, climatic and cultural differences.

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