Scientific article

Leaf area estimation of individual leaf and whole plant of chickpea (*Cicer arietinum* L.) by means of regression methods

Estimación del área foliar de hoja individual y planta entera en el cultivo de garbanzo (*Cicer arietinum* L.) mediante métodos de regresión

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Abstract

The quantification of the leaf area (LA) of a crop is important due to its relationship with solar radiation interception and the production of photoassimilates that are essential for plant growth. For this quantification, different LA measurement methods are available, and the choice to use one or the other depends on different factors. Nonetheless, information about methodologies for estimating chickpea LA is scarce. Hence, this paper aimed to select variables that allow estimating LA accurately in Norteño and Chañaritos S-156 cultivars, considering individual leaves and the whole plant. A completely randomized experimental design with 4 replications was used, in plots of six 13-meter-long lines at 0.52 m spacing, with a density of 26 plants/m^2. Individual leaf and whole plant LA data were obtained by processing photographs of each leaf with ImageJ 1.x. To estimate LA per leaf and plant, linear and nonlinear regression models were adjusted, and their performance was evaluated. The results showed that LA could be estimated on the basis of individual leaf and whole plant LA data using fresh leaf weight as a regression variable. For individual leaf LA, equations $y = 0.538 + 31.7831\,x$ and $y = -0.9508 + 10.4853\sqrt{x} + 10.9107(\sqrt{x})^2$ were selected for Norteño and Chañaritos S-156 cultivars, respectively. Regarding whole plant LA, equations $y = 42.4679 + 33.4606\,x$ and $y = 19.3918 + 36.5052\,x$ were chosen for Norteño and Chañaritos S-156 cultivars, respectively.

Keywords: ImageJ; Leaf area; Chickpea; Regression methods.

Resumen

Es importante cuantificar el área foliar (AF) de un cultivo debido a su relación con la intercepción de la radiación solar y la producción de fotoasimilados necesarios para el crecimiento. Para su determinación existen métodos de estimación o medición de AF, cuya elección dependerá de diversos factores. En garbanzo, la información sobre metodologías para estimar el AF es escasa. Por tal motivo, el objetivo del presente trabajo fue seleccionar variables que permitieran una adecuada estimación del AF por hoja y por planta entera en los cultivares Norteño y Chañaritos S-156. A tal fin se realizó un ensayo siguiendo un diseño experimental completamente aleatorizado con 4 repeticiones, en parcelas de 6 líneas distanciadas a 0,52 m y de 13 m de largo, con una densidad de 26 plantas/m^2. El AF por hoja individual y planta entera se obtuvo procesando las imágenes fotográficas de cada hoja con el software ImageJ 1.x. Para estimar el AF por hoja y por planta se ajustaron modelos de regresión lineales y no lineales. Se evaluó el desempeño de cada modelo de regresión propuesto. Los resultados obtenidos evidencian que se puede estimar el AF por hoja individual y planta entera utilizando como variable regresora el peso fresco de las hojas. En AF por hoja individual se seleccionaron las ecuaciones $y = 0{,}538 + 31{,}7831\,x$; $y = -0{,}9508 + 10{,}4853\sqrt{x} + 19{,}9107(\sqrt{x})^2$ para los cvs. Norteño y Chañaritos S-156, respectivamente. Para estimar el AF por planta entera se seleccionaron las ecuaciones $y = 42{,}4679 + 33{,}4606\,x$ para el cv. Norteño; $y = 19{,}3918 + 36{,}5052\,x$ para el cv. Chañaritos S-156.

Palabras clave: ImageJ; Área foliar; Garbanzo; Métodos de regresión.

Introduction

Quantifying leaf area (LA) in a crop is important, as it has a relationship with solar radiation interception and the production of photoassimilates essential for plant growth. It has been proven that LA is associated with vegetative plant growth, developmental rate and photosynthetic efficiency (Montoya Restrepo *et al*., 2017), and its determination helps to understand crop response to different experimental treatments (Bakhshandeh *et al*., 2010).

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LA can be measured or estimated through several methods, the election of which will depend on morphological leaf characteristics (maximum length, maximum width, rachis length), plant variables (height, number of nodes, number of leaves per branch), sample size, infrastructure and economic resources available, among other factors. Among the instruments available to measure or estimate LA, there are precise and widely used tools, such as plant canopy analyzers (LAI 2200C), area meters (LI-3100C) and LP-80 ACCUPAAR, but they are expensive and, in some cases, involve destructive methods. Alternatively, LA can be estimated using statistical models, which constitute economical and precise tools that imply measuring adequate variables (which depend on the crop) (Rahemi-Karizaki *et al.*, 2007; Bakhshandeh *et al.*, 2010; Olfati *et al.*, 2010; Garcés Fiallos and Forcelini, 2011; Jerez Mompie *et al.*, 2014; Urteaga, 2015; Interdonato *et al.*, 2015; Montoya Restrepo *et al.*, 2017; Téllez *et al.*, 2018). In certain crops, the differences in leaf and plant architecture attributable to cultivar characteristics require that LA be measured or estimated for each cultivar, or for cultivars of similar plant architecture (Toker *et al.*, 2012).

Chickpea is a cold season legume which grows mainly in arid and semiarid regions. It harbors symbiotic bacteria that fix atmospheric nitrogen, and when included in a rotation with cereals, chickpea brings about economic, environmental and agronomic benefits, apart from being a good source of proteins, minerals and vitamins for the human diet (Samineni *et al*., 2015). It is an herbaceous annual plant, with wide-spread primary, secondary and tertiary branches (Singh and Diwakar, 1995). Its leaf shape depends on the cultivar, and can be normal, simple or multi-pinnate (Toker *et al.*, 2012). It has been observed that normal leaf cultivars, compared to simple leaf cultivars, show a major individual LA and increased light interception (Li *et al.*, 2008), a larger photosynthetic surface, and a higher dry matter production (Li *et al.*, 2006). In Argentina, chickpea crop adapts very well to a wide range of agroecological conditions. Currently, in Argentina there are 6 chickpea cultivars available, with Norteño and Chañaritos S-156 being the first cultivars released and two of the most cultivated. These cultivars are widely accepted by farmers, as their high yield and great grain size result in good sales prices.

Reports on the evaluation of linear and non-linear regression models to calculate LA in chickpea are scarce (Soltani *et al.*, 2006; Rahemi-Karizaki *et al.*, 2007), and to the best of our knowledge, there are no national reports. In view of this, the aim of the present paper was to select variables which allow estimating individual leaf and whole plant LA adequately in two chickpea cultivars widely grown in Argentina.

Materials and methods

Agronomic management and experimental design

The field experiment took place in Finca el Manantial, Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán, in Tucumán province, Argentina (26º50'6.9'' S – 65º16'44.6'' W), which is located in the central subhumid-humid plain. This area has a subhumid-humid subtropical climate, with a dry season and a monsoon regime.

The experimental plots were sown on July 19, 2017, following a completely randomized experimental design with 4 replications. Each plot consisted of six 13-meter-long lines, spaced 0.52 m apart. Seeding density was 26 plants/m^2, and the seeds belonged to cultivars Norteño (with a seed weight of 59 g/100 seeds, 90 days to flowering, and a 150-170 day crop cycle) and Chañaritos S-156 (a 49 g/100 seed weight, 65 days to flowering, and a 140-150 day crop cycle), both of kabuli type and characterized by having a semierect growth habit, a normal leaf (odd-pinnate) shape, white flowers and medium sized cream colored seeds (Carreras *et al.*, 2016).

The seeds were treated with carbenzadim + thiram (625 cc/100 kg seed) and inoculated with *Mesorhizobium ciceri* (200 cc/50 kg seed). Herbicides (prometrine: 2.51/ha, and glyphosate: 2.5 1/ha), fungicides (azoxystrobin + difenoconazole: 0.45 1/ha) and insecticides (lufenuron + profenofos: 0.25 1/ha) were applied to prevent weed competition and ensure plant health.

Six samples were collected every 21 days during the crop cycle. Each sampling consisted in selecting one plant at random and removing it from the soil in all the experimental units. Sampling was repeated throughout the crop cycle so as to obtain highly variable measurements of the parameters borne in mind to estimate LA more precisely. The plants were extracted early in the morning and taken to the lab immediately, so as to avoid plant dehydration, and they were kept at 21°C for immediate processing.

Variables measured and LA estimation

Leaves from the sampled plants were cut off at their stem insertion and the stipules were discarded (Figure 1). Leaves were held flat between two glass plates (3 mm thickness) for maximum expansion, and then photographed with a 14 mpx digital camera. Senescent leaves and those with broken or missing folioles were not included in the study. With the digital photographs obtained, individual leaf area (LAl) (in cm^2), as well as maximum width (W), maximum length (L) and rachis length (Lr) (in cm), were obtained by processing the high resolution digital images using the ImageJ 1.x software, which calculates areas contrasting leaf tone in relation to the background (Schneider *et al.*, 2012; Di Benedetto and Tognetti, 2016). Fresh leaf weight (Flw) was determined by using a digital Mettler H80 scale (precision = 0.1mg).

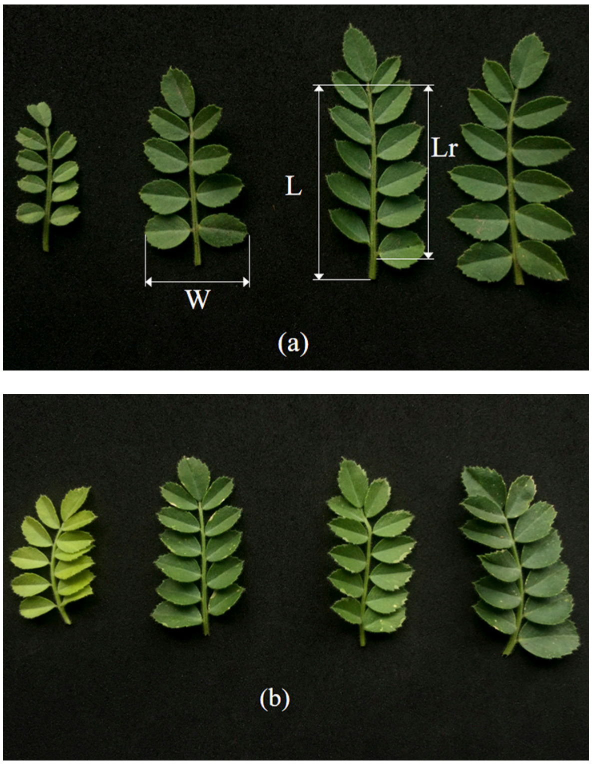

Figure 1. Different chickpea leaf sizes: (a) cv. Norteño, (b) cv. Chañaritos S-156. L: maximum length, Lr: rachis length, W: maximum leaf width.

A sample from the data provided by ImageJ of all the photographed leaves was used to obtain observed individual LA (LAl) mean value. The sampling was intended to cover a wide range of leaf sizes in order to obtain estimates with a greater predictive capacity. The variables used to estimate individual LA were W, L, Lr and Flw. Sample sizes, corresponding to number of leaves measured in the 6 sampling dates, were 489 for cv. Norteño, and 513 for cv. Chañaritos S-156. The value of each observed plant LA (LAp) was obtained by adding all the LAl of the leaves of each plant evaluated with the image processor mentioned previously. The variables used to estimate plant LA were total number of leaves per plant (N), fresh leaves per plant weight (Fpw), plant maximum height (H) and number of primary branches (B). Sample sizes were 23 plants for cv. Norteño, and 21 plants for cv. Chañaritos S- 156.

Model building

To estimate LA per leaf and plant, linear and non-linear regression models were adjusted (Table 1). As a first step, a correlation analysis between observed LAl, LAp, and leaf and plant variables (presented in Table 2) was made. Only variables showing a high correlation (r > 0.90) were considered when selecting regression models.

Table 1. Regression models used to estimate leaf areas of both individual leaves and whole plants (y) in chickpea.

Model	Equation
Linear	$y_{ij} = a + b\, x_i + e_{ij}$
Logarithm	$\log y_{ij} = a + b \log x_i + e_{ij}$
Polynomial	$y_{ij} = a + b_1 x_i + b_2 x_i^2 + e_{ij}$
Exponential	$y_{ij} = a\, x^{b}_{i} + e_{ij}$
Multiple linear regression	$y_{ij} = a + b_1 x_1 + b_2 x_2 + e_{ij}$

Models were selected with the following criteria: fulfillment of analysis assumptions (independent normal variables with expectation zero and constant variance residuals), coefficient of determination (R^2) greater than 0.90, lower root mean square error (RMSE) values, and model simplicity, according to the principle of parsimony (Crawley, 2013). The statistical analyses were run with Infostat software (Di Rienzo *et al.*, 2018).

Validation of the estimated models

Independent data of individual leaves and whole plants (Table 3) randomly selected from the material collected from the same field experiment on the same dates were used to validate the estimated models. LA was fitted using Flw and Fpw as regression variables in the equations that fit best.

To evaluate the adjustment of the selected estimated models, a linear regression analysis between LA observed in the validation data set and fitted LA values was run.The performance of each

Table 2. Variables used to generate regression models to estimate leaf area (LA) per leaf and for the whole plant in chickpea (cvs. Norteño and Chañaritos S-156).

LA	Cultivar	Variable	n	V_m(mean ± se)	V_{min}	V_{max}
Leaf	Norteño	Flw	489	0.120 ± 0.003	0.004	0.380
		W	489	1.901 ± 0.020	0.450	3.320
		L	489	3.110 ± 0.040	0.480	5.180
		Lr	489	2.550 ± 0.040	0.140	4.670
		LAl	489	4.360 ± 0.110	0.150	11.900
	Chañaritos S-156	Flw	513	0.108 ± 0.070	0.007	0.348
		W	513	1.934 ± 0.024	0.640	3.523
		L	513	2.468 ± 0.042	0.069	4.926
		Lr	513	2.468 ± 0.042	0.069	4.926
		LAl	513	4.441 ± 0.114	0.379	11.820
Plant	Norteño	N	23	135.76 ± 27.73	8.00	441.00
		Fpw	23	15.81 ± 2.05	1.00	31.00
		H	18	28.49 ± 3.49	8.35	59.35
		B	23	4.13 ± 0.29	2.00	7.00
		LAp	23	562.62 ± 118.03	13.40	1855.40
	Chañaritos S-156	N	21	196.25 ± 48.12	9.00	884.00
		Fpw	21	21.26 ± 5.41	0.22	100.16
		H	18	28.60 ± 3.42	7.11	49.56
		B	21	3.76 ± 0.31	2.00	6.00
		LAp	21	736.26 ± 173.77	8.12	3244.57

n: sample size, Vm: medium value, s.e.: standard error, Vmin: minimum value, Vmax: maximum value. Flw: fresh leaf weight (g); W: maximum width (cm); L: maximum length (cm); Lr: rachis length (cm); LAl: observed individual leaf area (cm^2); N: total leaf number per plant; Fpw: fresh leaves per plant weight (g); H: maximum plant height (cm); B: number of primary branches; LAp: total plant leaf area observed (cm^2).

proposed regression model was evaluated by considering the values of the refined index of agreement (dr) (Willmott *et al.*, 2012), R^2, RMSE and the regression coefficients, where values of b close to 1, and of a close to 0 are expected.

Results

The results of the correlation analysis are shown in Table 4. The variables which presented $r > 0.90$ were Flw, W, N and Fpw for both cultivars, and H only for cv. Chañaritos S-156. In all cases high levels of significance ($p < 0.001$) were obtained.

The equations with the best adjustments that derived from LA regression in each variable, together with R^2 and RMSE, are shown in Table 5. In all cases, the estimated regression coefficient b was significantly different from 0 ($p < 0.05$). Equations 1, 2, 6, 7, 8 and 3, 4, 9, and 10 correspond to regressions obtained with data from Norteño and Chañaritos S-156 cultivars, respectively. Equations 1, 3, and 5 were selected for individual leaf LA estimation in cv. Norteño, cv. Chañaritos S-156, and both cultivars, respectively. In these

Table 3. Variables used to validate the regression models generated to estimate individual leaf and whole plant LA in chickpea (cvs. Norteño and Chañaritos S-156).

LA	Cultivar	Variable	n	Vm ± s.e.	Vmin	Vmax
Leaf	Norteño	Flw	236	0.130 ± 0.005	0.007	0.395
		LAl	236	4.632 ± 0.166	0.267	12.476
	Chañaritos S-156	Flw	227	0.100 ± 0.004	0.010	0.290
		LAl	227	4.335 ± 0.155	0.221	11.575
Plant	Norteño	Fpw	13	18.783 ± 4.871	0.327	49.403
		LAp	13	732.278 ± 167.286	13.105	1630.225
	Chañaritos S-156	Fpw	11	15.741 ± 4.158	0.898	44.081
		LAp	11	570.900 ± 145.710	35.130	1524.150

n: sample size, V_m: medium value, s.e.: standard error, V_{min}: minimum value, V_{max}: maximum value. Flw: fresh leaf weight (g); Fpw: fresh leaves per plant weight (g); LAl: observed individual leaf area and LAp of whole plant used for validation of the estimated models (cm^2).

three cases the regression variable was Flw (individualleaf fresh weight), presenting a $R^2 > 0.90$.

Table 4. r value from the correlation analysis between observed LA and variables considered for individual leaves and whole plants in chickpea (cvs. Norteño and Chañaritos S-156).

LA	Variable	Cultivar	
		Norteño	Chañaritos S-156
Leaf	Flw	0.956***	0.964***
	W	0.914***	0.920***
	L	0.842***	0.848***
	Lr	0.837***	0.866***
Plant	N	0.989***	0.990***
	Fpw	0.993***	0.999***
	B	0.783***	0.923***
	H	0.551**	-0.068

*** indicates $p < 0.001$,** indicates $p < 0.01$. Flw: fresh leaf weight (g); W: maximum width (cm); L: maximum length (cm); Lr: rachis length (cm); N: total leaf number per plant; Fpw: fresh leaves per plant weight (g); H: maximum plant height (cm); B: primary branch number.

To estimate LA per plant, Equation 7, 10 and 11 were selected for cv. Norteño, cv. Chañaritos S-156, and both cultivars, respectively. In all cases, the regression variable was Fwp (fresh weight of total leaves per plant) with $R^2 \approx 0.99$, with the three selected equations correspondingto a linear regression.

Results from model validation (i.e. regression coefficient values, R^2, RMSE, and dr) are shown in Table 6. In the case of cv. Norteño, similar R^2 and RMSE values in Equations 1 and 5 could be observed. The dr coefficient was slightly closer to 1 in Equation 5. Coefficients a and b presented values closer to 0 and 1, respectively, in both equations. Similarly, cv. Chañaritos S-156 presented similar values of R^2, RMSE and a, but Equations 3 and 5 showed differences in b and dr. Coefficient b in Equation 5 presented a value higher than 1, which would cause LA to be overestimated with higher Flw levels. By contrast, Equation 3 presented a value closer to 1, keeping a proportioned relationship among LA and LA' values, with adequate dr values. In the equation analysis to estimate LA per plant, cv. Norteño presented similar R^2, RMSE, and dr values in Equations 7 and 11, in comparison to a and b values, which presented the closest values to 0 and 1, respectively, in Equation 7. Cv. Chañaritos S-156 showed similar R^2 values, but RMSE was lower, and dr was closer to 1 in Equation 11 than in Equation 10. Coefficients a and b showed closer values to 0 and 1, respectively, in Equation 11. In Figure 2, graphs a, b, c, and d show data validation.

Table 5. Estimated regression models with the best adjustments based on LA regression observed in each variable, when estimating individual leaf and whole plant LA (y).

LA	Cultivar	Variable	Eq.	Adjusted equations	R^2	RMSE
Leaf	Norteño	Flw	(1)	$y = 0.538 + 31.7831x$	0.915	0.698
		W	(2)	$\log y = -0.0414 + 2.2681 \log x$	0.844[1]	0.128
	Chañaritos S-156	Flw	(3)	$y = -0.9508 + 10.4853 \sqrt{x} + 19.9107 (\sqrt{x})^2$	0.936	0.669
		W	(4)	$y = 1.1845 x^{1.9075}$	0.856[1]	1.002
	Chañaritos S-156 + Norteño	Flw	(5)	$y = 0.6107 + 33.3317 x$	0.909	0.751
Plant	Norteño	N	(6)	$y = -183.2205 + 41.1373 \sqrt{x} + 2.1213 (\sqrt{x})^2$	0.973[1]	22.460
		Fpw	(7)	$y = 42.4679 + 33.4606 x$	0.991	15.987
		x_1=N x_2=Fpw	(8)	$y = -52.7641 + 2.9044 x_1 - 0.0043 x^2_1 + 0.0000023 x^3_1 + 20.4114 x_2$	0.994[1]	13.152
	Chañaritos S-156	N	(9)	$y = -30.7204 + 4.6152 \sqrt{x} + 4.0185 (\sqrt{x})^2$	0.977[1]	15.702
		Fpw	(10)	$y = 15.1421 + 38.3333 x$	0.998	5.058
	Chañaritos S-156 + Norteño	Fpw	(11)	$y = 19.3918 + 36.5052 x$	0.990	16.225
		x_1=N x_2=Fpw	(12)	$y = -15.4386 + 1.2481 x_1 - 0.0011 x^2_1 + 29.9290 x_2$	0.992[1]	14.224

R^2: determination coefficient, RMSE: root mean square error. All estimated parameters were significant ($p < 0.05$). Flw: fresh leaf weight (g); W: maximum width (cm); N: total leaf number per plant; Fpw: fresh leaves per plant weight (g). [1]Discarded for not complying with the requirements mentioned in the Model building section.

Table 6. Validation of the regression models generated to estimate LA per individual leaf and for the whole plant. Estimated values ± standard error.

LA	Cultivar	eq.	y = a + bx		R2	RMSE	dr
			a	b			
Leaf	Norteño	(1)	-0.037*	1.003 ± 0.019	0.921	0.714	0.864
	Chañaritos S-156	(3)	-0.368 ± 0.091	1.091 ± 0.019	0.936	0.618	0.875
	Norteño	(5)	-0.081*	0.957 ± 0.018	0.921	0.780	0.934
	Chañaritos S-156	(5)	-0.561 ± 0.091	1.214 ± 0.020	0.940	0.760	0.935
Plant	Norteño	(7)	61.470*	1.000 ± 0.070	0.944	144.888	0.890
	Chañaritos S-156	(10)	6.614*	0.912 ± 0.020	0.995	71.765	0.939
	Norteño	(11)	86.158*	0.916 ± 0.064	0.944	143.610	0.902
	Chañaritos S-156	(11)	1.822*	0.958 ± 0.021	0.995	43.204	0.961

* indicates no significant difference from 0 ($p < 0.05$).
eq: equation number as presented in Table 4; Eq Adj: adjustment equation, with y corresponding to LA validation values (LAl' or LAp'), and x corresponding to fitted LA values (LA'); a and b: estimated linear regression coefficient; R^2: determination coefficient; RMSE: root mean square error; dr: refined index of agreement.

Discussion

Fresh leaf weight was the variable that showed the best adjustment in the chickpea cultivars under study. This result contradicts previous reports of linear leaf measurement being a LA predictive parameter in tomato (Astegiano *et al*., 2001), soybean (Bakhshandeh *et al*., 2010), cabbage (Olfati *et al*., 2010), bean (Bhatt and Chanda, 2003), pepper (Téllez *et al*., 2018), broad bean (Peksen, 2007), potato (Jerez Mompie *et al*., 2014), walnut tree (Keramatlou *et al*., 2015) and fig (Urteaga, 2015).

Maximum leaf width also showed a good LA

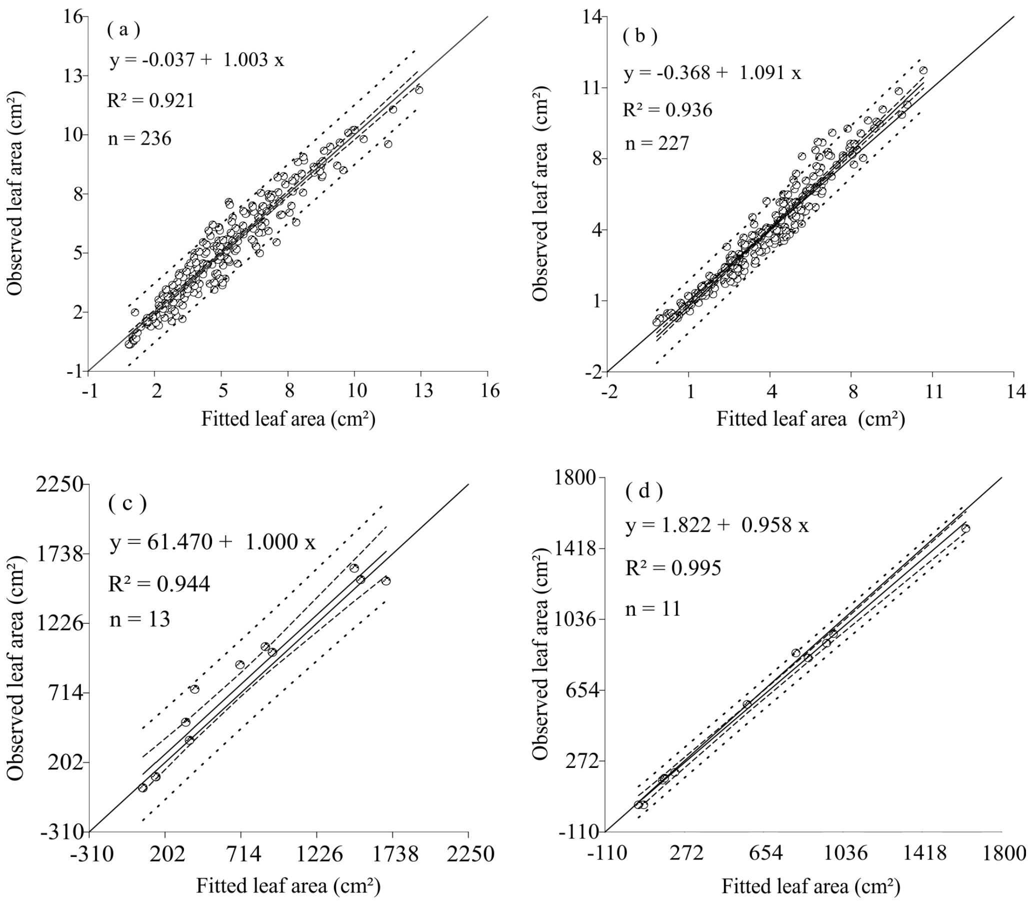

Figure 2. Adjustment between observed LA and fitted LA for individual leaves in: cv. Norteño (a), Chañaritos S-156 (b); and for whole plants in: cv. Norteño (c) and Chañaritos S-156 (d). Fitted values were calculated using validation data. The adjustment equation, regression coefficients and number of samples are presented. The continuous lines correspond to the better adjustment equation, the dashed lines to the 95% confidence intervals, and the dotted lines to the 95% prediction limits. The line through the origin corresponds to line 1:1.

predictive power ($R^2 \approx 0.85$). However, this variable is not adequate to estimate LA, since measuring small leaf widths (0.45 cm Norteño and 0.64 cm Chañaritos S-156) is both difficult and time-consuming.

Since maximum leaf length and rachis length did not show an acceptable correlation, they could to be considered as variables to estimate individual leaf LA precisely, and as with maximum leaf width, measuring them would also take time.

Among the variables considered to estimate LA per plant, total green leaf fresh weight was the variable with best fit, also leading to anaccurate estimation. In agreement with this, Garcés Fiallos and Forcelini (2011) suggest using fresh or dry weight as a variable in estimating LA per plant in soybean.

Plant height was not a good basis for plant foliar area estimation, as opposed to what Rahemi-Karizaki *et al.* (2007) found.This discrepancy may be due to the fact that these authors worked on different cultivars, and on plants that were shorter and that had fewer branches.

A low correlation between primary branch number and LA per plant was found in this paper. This lack of correlation may be explained by the observed variation in branch number, which is dependent on inter-plant competence (Siddique *et al.*, 1984; Peñaloza and Levio, 1991).

As cited by Soltani *et al.* (2006), total leaf number can be used as a predictive LA variable in chickpea cultivars. Likewise, Montoya Restrepo *et al*. (2017) found an adequate relationship between leaf number in a branch and their LA in a coffee cultivar. However, as chickpea cultivars may present minimum foliar dimensions (0.45-3.2 cm) and a high number of leaves (400-800), estimating LA by using this variable would be a difficult and arduous task. Conversely, this variable is more suitable for crops like sorghum, as reported by Interdonato *et al*. (2015), who propose a model for predicting LA per plant in a sorghum cultivar using plant height and green leaf number as regression variables.

Conclusions

Using fresh leaf weight as a regression variable constitutes a low cost and highly precise method for estimating LA per leaf and individual plant in chickpea.

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