

Equations for estimation of the foliar area of *Coffea canephora* genotypes

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ABSTRACT. Knowledge of the leaf characteristics of the coffee tree, such as leaf dimensions, is of great importance for management of this crop, since it directly impacts on plant development. We evaluated the genetic diversity of 43 *Coffea canephora* genotypes and developed and compared mathematical models for estimating the leaf area of distinct genotypes using linear characteristics. Leaves from 2½ year old trees were collected from the upper middle third of the plant and the length of the central vein and maximum width of the leaf were measured; the leaf area was subsequently measured to determine real leaf area (RLA). The variables leaf length (L), leaf width (W), RLA and length x width (LW) were subjected to Pearson correlation analysis and grouped by the Tocher optimization method. All combinations were tested by linear models according to the measured parameters, and for each model R² was adjusted and Bayesian information criterion tested. After choosing the variable, equations were defined considering two parameters, which were

subjected to cross-validation by comparing between observed x predicted areas. The 43 genotypes formed three groups according to the Tocher procedure, wherein one group was comprised of 41 genotypes. High Pearson linear correlations were found between LW x RLA (0.99), followed by W x RLA (0.95), and as such, LW best estimated the coffee leaf area; but the variable width can also be adopted, with greater ease of field measurement. The equations designed including both variables were significant at 1% and 0.1% according to the F test, and cross-validation analysis confirmed the adjustment of the equations, with equal or very similar values.

Key words: Conilon coffee; Leaves; Linear measurements; Non-destructive method

INTRODUCTION

It is believed that photosynthesis is the most important photochemical reaction, through which light is converted into biological energy in plants (Wang et al., 2018). Leaf area is fully related to this process, as photosynthetic efficiency depends on the rate of photosynthesis per unit of leaf area and on the interception of solar radiation, which is influenced by the canopy architecture and by the size of the photoassimilator system (Favarin et al., 2002; Partelli et al., 2006; Fascella et al., 2013; Guimarães et al., 2013); consequently leaf area is characterized as one of the most important parameters in the evaluation of plant growth and yield.

Knowledge about leaf area has various agronomic applications, such as estimation of water loss through transpiration and choice of the appropriate irrigation method according to the plant water requirement; selection of the cultivar, area of planting and the management used in the crop; dosage calculations for foliar product applications such as fungicides, insecticides and fertilizers, stipulation of biotic and abiotic damage; quality and final crop yields (Partelli et al., 2006; Busato et al., 2010; Espindula et al., 2018).

Measurement of leaf area can be done directly in a destructive or non-destructive manner, and indirect in a non-destructive manner (Partelli et al., 2006, 2007; Busato et al., 2010; Guimarães et al., 2013). In order to determine the leaf area directly, the leaves are collected from the plant, which characterizes this method as destructive and of high labor. On the other hand, non-destructive direct measurement is simpler, but requires expensive equipment, whereas non-destructive indirect methods allow for successive evaluations in the same plant and agility in evaluations, as well as low cost and low labor demand that is not required to be specialized (Busato et al., 2010; Ilkaee et al., 2011; Toebe et al., 2012; Schmidt et al., 2014).

The indirect non-destructive method is based on the estimation of the leaf area by measuring the width and length parameters of the leaf while still on the plant (Schmidt et al., 2016; Espindula et al., 2018), from which is possible to utilize statistical models. The regression equations between the real leaf area (RLA) and the dimensional parameters of the leaves have good precision and low cost, eliminating the need for expensive measurement tools or performing lengthy geometric reconstructions (Schmidt et al., 2014; Brinate et al., 2015ab).

A study by Partelli et al. (2006) proposed an equation model for conilon coffee (*C. canephora*) considering the length of the central vein, generating two equations according to the age of the plant, be it young or mature. However, due to the great genetic variability in *C. canephora* (Covre et al., 2016; Espindula et al., 2018) the plants from a specific population are heterogeneous and have distinct characteristics, and as such the revalidation of these methods becomes necessary after some time. However, the wide availability of new genotypes and their numerous distinct morphological characteristics can change the precision of the methods based on the leaf area of older genotypes (Brinate et al., 2015ab). Espindula et al. (2018) proved this by testing the efficiency of the equation developed by Partelli et al. (2006) on 15 new genotypes of robust coffee trees, concluding that they should be adjusted for each genotype due to their dissimilar leaf characteristics.

The shape of the leaf is an individual morphological feature that is dependent on the relation between length and width and the amount of cutouts at the edge of the leaf limb, so it is necessary to elaborate specific models of leaf area estimation for each plant species (Cargnelutti et al., 2015) and even within the same species for different cultivars. In addition, each leaf, depending on the degree of differentiation or species, has the ability to react to changes in its habitat, altering metabolism, anatomy and morphology in order to maintain photosynthetic efficiency (Rodrigues et al., 2016; Martins et al., 2016; Espindula et al., 2018).

Given this context, this study aims to analyze the foliar genetic diversity of 43 coffee genotypes and to obtain mathematical equations, namely, a correction factor that allows estimating the leaf area of the different genotypes through linear dimensional parameters of the leaves.

MATERIAL AND METHODS

The experiment was conducted in a field with 43 *C. canephora* genotypes selected by coffee growers from the region (Table 1). Planting occurred in April 2014 in the municipality of Nova Venécia, northern region of the state of Espírito Santo, Brazil, on a private property located at latitude 18°66'23" south and longitude 40°43'07" west, altitude of 50 m; annual average temperature is 23°C. The region has a tropical climate, characterized by a hot and humid summer and a dry winter, classified as Aw according to Köppen (Alvares et al., 2013).

The genotypes were arranged in a randomized block experimental design, with three replications, each treatment having different genotypes, and each experimental unit containing seven plants. The planting was carried out with spacing of 3 m between rows and one meter between plants, which is equivalent to 3333 plants per hectare. Of the 43 genotypes, 42 were propagated by cutting and one by seed (Semente) (Table 1). The plants were conducted with four orthotropic stems and the whole experimental area was irrigated with a drip system.

To determine the leaf area, of the third or fourth leaves of plagiotropic branches located in the middle third of the plants were collected, with a total of 20 leaves per genotype. The leaves were conditioned in bags that were duly identified and sent to the laboratory. Afterwards, the the green leaves were measured using a ruler, measuring the length of the central vein and the maximum width of the leaf blade in centimeters (Partelli et al., 2006). Then, measurement of leaf area (LA) was made using a leaf area meter LI-

3100, LI-COR, Lincoln, NE, USA. These evaluations were carried out at two distinct times, October 2016 and February 2017.

Table 1. Identification of the 43 genotypes of *Coffea canephora*. Nova Venécia-ES.

Identification	Name	Identification	Name	Identification	Name
1	Verdim R	16	Pirata	31	Cheique
2	B01	17	Peneirão	32	P2
3	Bicudo	18	Z39	33	Emcapa 02
4	Alecrim	19	Z35	34	Emcapa 153
5	700	20	Z40	35	P1
6	CH1	21	Z29	36	LB1
7	Imbigudinho	22	Z38	37	122
8	AD1	23	Z18	38	Verdim D
9	Graudão HP	24	Z37	39	Semente
10	Valcir P	25	Z21	40	Emcapa 143
11	Beira Rio 8	26	Z36	41	Ouro negro 1
12	Tardio V	27	Ouro Negro	42	Ouro negro 2
13	AP	28	18	43	Clementino ^T
14	L80	29	Tardio C	-	-
15	Bamburral	30	A1	-	-

Genotype 33, 34 and 40 belong to Emcapa variety 8131; genotypes 1, 11, 15, 16 and 30 belong to Tributum variety (Giles et al., 2018); and 30 and 35 to cv. Andina (Partelli et al., 2019).

The foliar variables: length (L) in cm, width (W) in cm, real leaf area (RLA) in cm² and length x width (LW) in cm² were subjected to Pearson's linear correlation analysis. The generalized distance matrix of Mahalanobis (D²) was adopted as measure of dissimilarity for the genetic diversity analysis, and the clustering was performed with use of the Tocher optimization method. Afterwards, all combinations of linear models were tested according to the existing parameters, the R² (Coefficient of determination) was adjusted, and BIC (Bayesian Information Criterion or Schwarz Criteria) was tested for each model, being used to set up a plot diagram. After selecting the variable that best estimated the leaf area considering the correlation coefficients, the statistical equations/mathematical models were defined by considering the parameters β_0 and β_1 , according to the Tocher clustering. The cross-validation of the model was performed in accordance with the model selected by comparing the value for observed area x predicted area. All analyses were performed using the R programming language (R Core Team, 2018) and the packages leaps (Lumley, 2017) and MASS (Venables and Ripley, 2002).

RESULTS AND DISCUSSION

Initially the leaf measurements were carried out at two different times as specified in the materials and methods; however, when proceeding with the analyses, it was found that the results obtained for both evaluations were very similar for Pearson's linear correlation, Tocher clustering, Coefficient of determination and BIC, and as such we opted to work with unified data instead of separately.

Pearson's correlation analysis was performed to determine the degree of relationship between the measured variables (Table 2). The correlation study provides the information that the traits are genetically associated to each other (Bikila and Sakiyama, 2017), allowing us to evaluate how much the change in a variable can affect correlate variables. Positively

correlated variables indicate that both either benefit from or are harmed by the same causes of environmental variations, correlations with negative values indicate that the environment favors one character and work against the other (Silva et al., 2016).

Table 2. Linear Pearson correlation between leaf length (L), width (W), real leaf area (RLA) and length x width (LW) of 43 coffee genotypes.

	Length (L)	Width (W)	Real Leaf Area (RLA)	LW
Length (L)	1	0.63	0.82	0.85
Width (W)	**	1	0.95	0.94
Real Leaf area (RLA)	**	**	1	0.99
LW	**	**	**	1

** are significant at 1% probability by the t-test.

Devore (2006) ranks correlation values from 0.00 to 0.19 as very weak; 0.20 to 0.39 as weak; 0.40 to 0.69 as moderate; 0.70 to 0.89 as strong; and 0.90 to 1.00 as very strong. It is noted that all correlation coefficients showed positive and strong association. Espindula et al. (2018) also found a positive correlation at a magnitude of 0.60 to 0.94 between the estimated and actual leaf areas for 15 robust coffee genotypes.

The correlations ranged from 0.63 to 0.99, with the lowest occurring between leaf length and the other parameters (0.63 to 0.85). The largest occurred between RLA and the LW product (0.99), followed by leaf width versus leaf area and LW (0.95 and 0.94) (Table 2). This demonstrates a possible association of the estimation of real leaf area by measuring the length and width (LW) with the measurement based on the equipment usually adopted and with a high degree of precision.

As the proposal of this study is to reduce the need for manpower and not to destroy the samples, the measurement of leaf width is probably effective for determination of the leaf area of the distinct genotypes, since the correlation value between width and RLA was 0.95, being quite satisfactory and efficient. Differently, Partelli et al. (2006) who obtained Pearson's linear correlation coefficients that were higher for leaf length than for leaf width, but corroborate with the higher correlation coefficients between leaf area and LW. Such differences observed between the respective studies may be related to how this study analyzed different genotypes, whereas the study by Partelli and collaborators has made estimates based on only one genetic material. Thus, it can be empirically reported that the variation between the genotypes is related more to the length than the width of the leaf; that is, different lengths for the different genetic materials being evaluated, an important answer that should be further analyzed in further studies as a form of differentiation between cultivars and their respective field identifications.

To examine the similarity and clustering of the genotypes with the least genetic divergence concerning the leaf characteristics estimated, we proceeded with the Tocher optimization method (Table 3), which is based on the formation of the first group with the pair of individuals with the highest similarity based on the dissimilarity matrix calculated by the Mahalanobis method. From this, the possibility of including new individuals is examined, adopting the criterion that the mean intragroup distance should be smaller than the mean intergroup distance (Cruz et al., 2011).

Table 3. Clustering of 43 genotypes of *Coffea canephora* with use of the Tocher optimization method based on leaf measurements.

Group	Genotypes														
1	1	3	5	6	7	8	9	10	11	12	13	14	15	16	17
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
			33	34	35	36	37	38	39	40	41	42	43		
2								2							
3								4							

The genotypes were divided into three groups, with 41 genotypes in group 1, and the other two groups were composed of genotypes 2 and 4, respectively. The Tocher clustering method was employed by Fonseca et al. (2006) when evaluating 32 clones constituting three clonal varieties forming three groups; also by Ivoglo et al. (2008) with 21 progenies of half-brothers divided into four groups; and by Covre et al. (2016) and Giles et al. (2018) with 34 and 30 promising genotypes, in which they obtained eight and three groups, respectively.

However, most genotypes (41 in all) were concentrated within one group (G1), it is important to note that this discrimination between groups of more homogeneous genotypes within the groups is necessary, since *C. canephora* genotypes present phenotypic dissimilarity, differing in growth, morphology, leaf characteristics and crown. Therefore, clustering them as to differences in leaf area may increase the efficiency of the proposed models to study their leaf area (Brinate et al., 2015a). Therefore, genotypes two and four are quite divergent from the others, since they were kept in isolated groups, demonstrating that there is genetic variability within the studied population, this is a remarkable property of the *C. canephora* species due to their alogamy reproduction mode and their autoincompatibility between some alleles. The other 41 genotypes that remained in the same group are similar for leaf characteristics, which contributes to obtaining a single model for leaf area prediction for the population, and this model must have a highly precise estimation of the desired parameters.

Based on the evaluated characteristics, all combinations of models were tested according to the existing parameters, adjusted to R^2 (Coefficient of determination) and tested for BIC (Bayesian Information Criterion or Schwarz Criterion) for each model. This process was performed considering the Tocher clusters as well as the 43 genotypes as a whole (Figure 1). The coefficient of determination (R^2) is the adjustment measure from a generalized linear statistical model, such as linear regression, in relation to the observed values, with parametric space between 0 and 1, indicating in percentage how much the model was adjusted to the observed values and explaining the estimates for each parameter. The higher the R^2 , the more explanatory the model is, and the better it conforms to the sample (Charnet et al., 1999; Levin et al., 2000). The Schwarz Bayesian Criterion (BIC) assumes the existence of a "true model" that describes the relationship between the dependent variable and the various explanatory variables among the various models under selection. It is then defined as the statistic that maximizes the probability of identifying the true model among the evaluated ones. The model with lowest BIC, that is, the more negative one, is considered the best fit model (Schwarz, 1978).

In addition, the selection of the model with the same BIC values is chosen as the model with the lowest degree of parameterization, that is, with less indirect cause characters associated with the direct effects parameters. When evaluating Figure 1 where the values of R^2 and BIC are found for all tested contrasts, the resultant LW product variable was the one that best represented the leaf area estimation (Figure 1), and when estimated without clustering (total) presented R^2 of 0.95 and BIC -1900, for cluster 1 (G1) the value for R^2 was 0.94 and BIC -1600, for cluster 2 (G2) the value for R^2 was 0.92 and BIC -65, and for cluster 3 (G3) the value for R^2 was 0.91 and BIC -64.

The adoption of the variable LW predominates in the best adjustments compared to the others, with more satisfactory R^2 and the lowest BIC. It is possible to have higher R^2 values and more negative BIC, i.e. a more adjusted equation, but for this it has to be considered more variables, which makes an equation super parameterized, that is, it needs a higher number of parameters to obtain a higher degree of reliability. In addition, it makes work less practical and efficient. These results corroborate Schmidt et al. (2014) who, when studying the leaf area of Catuaí and Catuaí red varieties, verified that the equations obtained with linear models and circumscribed from LW showed the highest R^2 values. Similarly, Antunes et al. (2008) concluded that the best fit is acquired by using the power method, based on measures of LW as an independent variable.

For pigeon pea, Cargenelutti Filho et al. (2015) found that the quadratic, power and linear models of Y as a function of the product of the length and width for the central leaflet limb ($LW \times CLL$) are adequate for the estimation of the leaf area determined by digital photos. The leaf area of the eggplant was better estimated by the models that used the measures of length and width (Hinnah et al., 2015).

If we consider only the leaf width variable, we obtain values of R^2 adjustments of 0.87, 0.84, 0.82 and 0.86, and BIC of -1300, -1000, -44 and -51, for "all", G1, G2 and G3, respectively. These are not considered the highest coefficients and most negative BIC; as reported above, the estimation of LW provides better adjustment, but given the difficulty of evaluating many parameters in the field, it may be the most viable alternative since it is faster and has the best cost benefit.

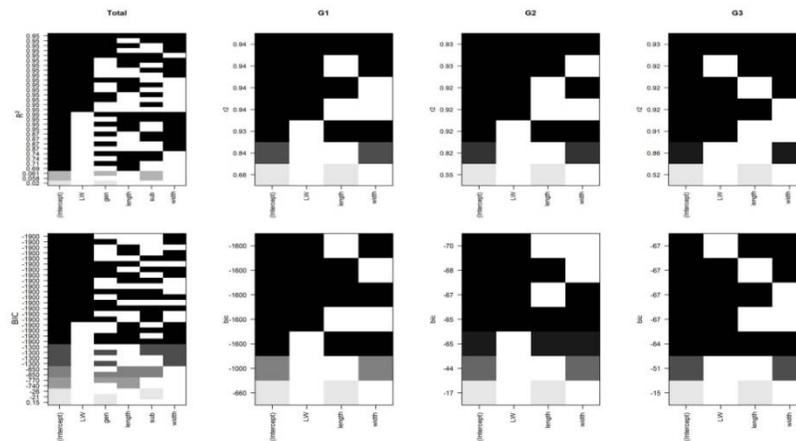


Figure 1. Value of R^2 (Coefficient of determination) and BIC (Bayesian Information Criterion) for the different models for coffee leaf measurements (length x width (LW), length and width).

For snap beans (*Phaseolus vulgaris*), Toebe et al. (2012) reported that the quadratic and power models as a function of the central leaflet width are adequate to estimate the complete leaf area obtained through the method of digital photos. Similarly for sunflower, Maldaner et al. (2009) found that leaf width better estimated leaf area, and concluded that this variable is subject to a smaller experimental error than leaf length. Both report that models using only the length (L) or the width (W) are preferable since they use only one of the dimensions of the sheet. As such, there is a 50% reduction in the number of linear dimension measurements to be performed in the field.

After selecting the LW and Width variables that best estimated the leaf area of the respective genotypes by means of determination coefficients and BIC, the equations were generated considering β_0 and β_1 according to the groups formed by the Tocher clustering method, but an equation was also defined without the clusters designated as total, considering the product LW and only width (Table 4).

Table 4. Equations prepared from the coffee leaf variables determined by the R^2 (coefficient of determination) and BIC (Bayesian Information Criterion) values according to the clusters formed by the Tocher optimization method.

Variables	clusters	Equations
Length x Width	Total	$Y = -0.815124 + 0.635626 \times LW^{***}$
	1	$Y = -0.550712 + 0.632022 \times CL^{***}$
	2	$Y = -1.0414 + 0.6425 \times CL^{***}$
	3	$Y = -5.62625 + 0.67793 \times CL^{***}$
Width	Total	$Y = -26.5722^{***} + 13.2743 \times W^{***}$
	1	$Y = -26.2319^{***} + 13.2754 \times W$
	2	$Y = -11.9575^{**} + 9.2588 \times W^{***}$
	3	$Y = -40.046^{***} + 14.735 \times W^{***}$

** , *** significant at 1% and 0.1% according to the F test.

Following the criteria mentioned above, in total, eight different mathematical models were generated; all of them gave significant results. As can be seen, the equations generated without the Tocher clusters were also significant and very similar to cluster 1 covering 41 genotypes. This demonstrates that using this equation to estimate the leaf area of all genotypes can be efficient and safe, while maintaining the possibility of discarding the clusters.

We then proceeded to compare the predicted and observed values for a better understanding of the precision in estimating the equation through the adoption of these parameters. Considering the variable LW, the highest values of the coefficient of determination (R^2) are found, these being 0.95, 0.93, 0.92 and 0.91 for "total" that considers the 43 genotypes, clusters 1, 2 and 3, respectively (Figure 2 and Table 5). A positive correlation was also found between the estimated and real leaf areas for all genotypes studied by Espindula et al. (2018), the values of r ranged from 0.60 to 0.94.

Considering the variable width, there were values for the coefficient of determination (R^2) of 0.87, 0.84, 0.82 and 0.86 for "all", clusters 1, 2 and 3, respectively (Figure 3 and Table 5). Logically they are lower than when considering the product Length x Width (Figure 2); however as already stated above, in the field situation it is much simpler to measure only one variable as in the case of width, and so R^2 being between 0.80 to 0.90 is accurate and can satisfactorily estimate the leaf area of said evaluated materials.

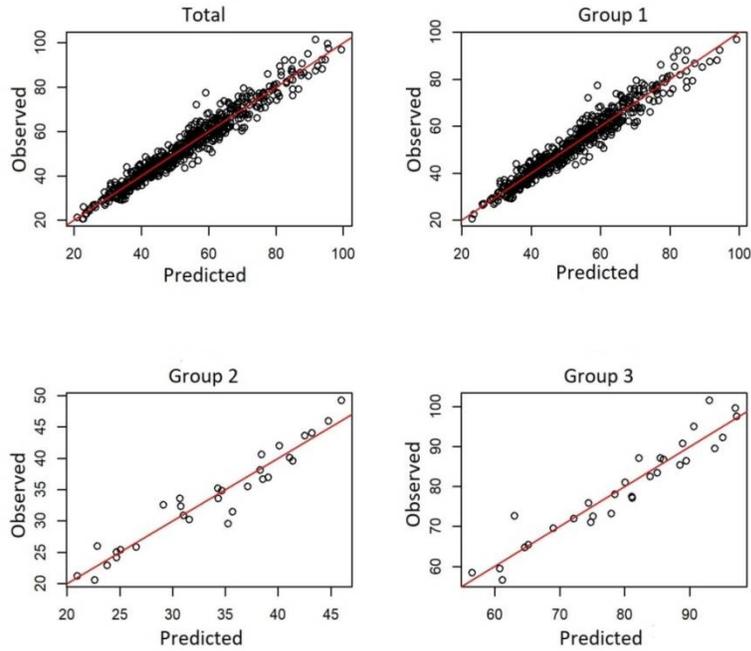


Figure 2. Relationship between predicted vs. observed value of coffee leaf area considering the product length x width (LW), following the groups formed by the Tocher method.

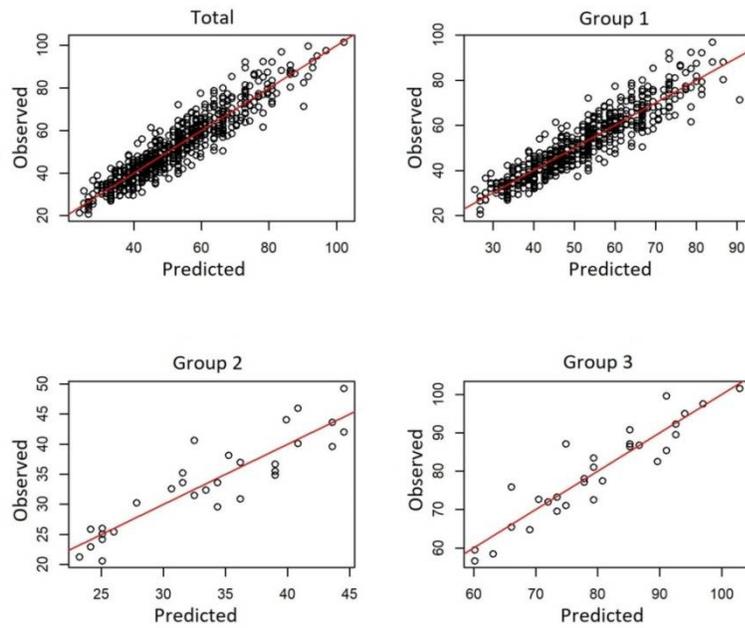


Figure 3. Relation between predicted value vs. observed value for coffee leaf area considering the variable leaf width (W), following the groups formed by the Tocher method.

To test the consistency of the determination coefficients, these were subjected to cross validation, which is a technique for evaluating the generalization capacity of a model. This technique is widely used in situations where the purpose of modeling is prediction (Martens and Naes, 1996). This model was then tested for accuracy in practice, i.e. its performance for a new set of data (Table 4). Cross-validation provided values very close to the original ones and even higher in cases such as clusters 1, 2 and 3. These results demonstrated the accuracy in estimating the leaf area of the genotypes by measuring the LW and W variables and elaborating the respective mathematical equations.

Table 5. Coefficient of determination (R^2) and cross validation considering coffee leaf length x width (LW) and width (W).

	Total	G1	G2	G3
R^2				
LW	0.95	0.94	0.92	0.91
Width	0.87	0.84	0.82	0.86
Cross Validation				
LW	0.95	0.94	0.94	0.94
Width	0.86	0.86	0.86	0.86

Given the high R^2 values when not considering clusters, it may not be necessary to study the leaf area of the genotypes separately, since the higher the value of R^2 (closer to 1) the more adjusted is the adopted model. Brinate et al. (2015) reported that even with differences between genotypes, the leaf shape pattern remains similar, confirming the applicability of the method to estimate leaf area using linear leaf dimensions for improved genotypes without occurring loss of accuracy, both for Arabica and Conilon coffee.

CONCLUSIONS

The genotypes had genetic variability, forming distinct clusters according to the Tocher method. Better correlations were found between LW and leaf area, followed by leaf width and real leaf area. The product between linear measures of length and width (LW) of the leaf limb satisfactorily estimated the coffee leaf area; but it is also possible to only adopt the width variable, which has greater ease of measurement in the field. The comparison between predicted and observed values demonstrated the adjustment of the equations, a fact proven by cross validation.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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