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# Applicability of the Method of Linear Dimensions to Estimate Leaf Area in Improved Genotypes of *Coffea arabica* and *Coffea canephora*

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## Abstract

**There are different methods to determinate leaf area in coffee plants; however, methodologies that allow measurement of leaf area accurately and in nondestructive ways are very important, as they are less economically costly and enable measurements on the same leaf over time, making it possible to describe accurate patterns of growth. The objective of this study was to investigate the applicability of the method to estimate leaf area using linear dimensions of leaves for improved genotypes of *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner. The experiment was conducted in two separated competition fields, each one following factorial schemes 10 × 2, with 10 genotypes and 2 methods to obtain the leaf area: measuring linear dimensions, and using equation model (estimated leaf area) and leaf area integrator (real leaf area). The genotypes for both *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner have different leaf areas, making it possible to discriminate groups of genotypes for both species using the size of their leaves as classification criteria. Even with the differences between genotypes, the pattern of leaf shape remains similar, confirming the applicability of the method to estimate leaf area using linear dimensions of leaves for improved genotypes, such as the model proposed by Barros, without the occurrence of loss of accuracy for the improved genotypes, for both Arabica and Conilon coffee, studied in this experiment.**

## Keywords

**Arabica Coffee, Conilon Coffee, Biometry, Leaves, Photosynthesis**

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# 1. Introduction

Regarding the cultivation of coffee, Brazil stands out as the world's largest producer of this commodity, which is one of the most valuable primary products traded worldwide. This country produces both Arabica coffee (*Coffea arabica* L.) and Conilon coffee (*Coffea canephora* Pierre ex A. Froehner) [1]. For years, the value of coffee is only surpassed by the value of fossil oils, being an important source of currency for developing countries and creating jobs for millions of people around the world. In addition, coffee is traded on the major stock exchanges worldwide, such as in London and New York [2].

Due to the importance of its cultivation to the world economy, studies related to coffee production have great relevance, especially for many developing countries that cultivate it. In this context, studies regarding leaf area of coffee plants are useful, since it is possible to gather early information that is normally correlated to the crop yield. Furthermore, analyses of plant growth, considering the increase of leaf area, have been employed in order to explain differences caused by genetic sequences or environmental changes [3], also being a tool to identify promising genotypes [4].

The morphological characteristics of leaves of the two widely grown species of the genus *Coffea* (*C. arabica* and *C. canephora*) are very similar. However, leaves in the same stage of development from those two species generally present different sizes and capacities to capture light. In addition, variations in the values of photosynthetic rates, transpiration and stomatal conductance have been reported, which are generally higher for Conilon coffee [5]. Greater total leaf area implicates on higher capacity to intercept solar radiation, which may result in higher photosynthetic rates, highlighting the importance of measuring this growth variable [6].

The anatomy and coffee leaf morphology demonstrates plasticity to environmental factors such as radiation [7] [8], water status [9]-[11] and nutrition [12]-[15]. Thus, it is important to know the coffee leaf traits to understand various physiological processes in the plant.

There are different methods to determinate leaf area in coffee plants; however, the indirect method of measuring linear dimensions and the direct method of using leaf area integrators are most widely used in scientific researches.

Estimates of leaf area based on mathematical equations, using linear measurements of dimensions of the leaf surface, are widely used to study leaf area in several plant species. For coffee plants, Barros *et al*. [16] described a methodology that follows a principle widely used in agricultural research, showing good precision, easing to execute and being nondestructive. Methodologies that allow measurement of leaf area accurately and in nondestructive ways are very important, as they are less economically costly and enable measurements on the same leaf over time, making it possible to describe accurate patterns of growth [17].

However, methods of measurement of leaf area using linear dimensions are normally based on samples of plants from one or very few genotypes of the studied species, creating the need to validate the equation models when the pool of relevant genotypes changes for that species. This fact is especially important when studying the species that present high genetic variability, which may lead to differences on leaf morphology between genotypes. Different studies have already described genetic variability for many traits in *C. arabica* [18] [19], while *C. canephora* is known by its higher variability [20] [21] due to its self-incompatibility [22].

For Arabica coffee, methods to estimate leaf area using the leaf dimensions are valid and widely used [16] [23]-[27], but many of the studies and validations were performed in genotypes from previous generations of the breeding programs. As new processes of breeding and selections are performed, some morphological traits of the plants may be influenced, making it necessary to check the accuracy of the evaluation techniques [28]. For Conilon coffee, especially due to its high genetic variability, the methods are still incipient.

The objective of this study was to investigate the applicability of the method to estimate leaf area using linear dimensions of leaves for improved genotypes of *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner.

# 2. Materials and Methods

## 2.1. Experimental Setup

The experiment was conducted in two separated competition fields, each one in a region that is considered adequate to the cultivation of each of the species of coffee. Both fields were located in the municipality of Alegre, southern Espírito Santo state, in the southeast region of Brazil, where the cultivation of coffee is one of the main economic activities.

The competition field with genotypes of *Coffea arabica* L. was located in the region of mountains, with geographic coordinates 20˚45'S and 41˚33'W and altitude of 690 m. The location presents an average annual temperature of 22˚C and average rainfall of between 1500 mm per year, with the rainy season from October to April and the dry season from May to September.

The competition field with genotypes of *Coffea canephora* Pierre ex A. Froehner was located at geographic coordinates 20˚45'S and 41˚29'W, in altitude of 138 m. The location has climate characterized as hot and humid in summer, and dry in the winter, with an average annual rainfall of 1200 mm and annual mean temperature of 24˚C.

The agricultural practices, such as fertilization, weed control and phytosanitary management, were established in accordance with those normally employed in the region, according to their need and following the current recommendations for the cultivation of Arabica coffee [29] or Conilon coffee [30] in Brazil.

The studies for each species of coffee were performed with factorial schemes 10 × 2, with 10 genotypes and 2 methods to obtain the leaf area. Both fields followed randomized block designs, each one with 10 genotypes of the proper species of coffee, with five plants per experimental plot and ten replications.

## 2.2. Genotypes

Ten genotypes of *Coffea arabica* L., originated from the breeding programs of institutions that are references in the development of cultivars of Arabica coffee, such as Instituto Agronômico de Campinas (IAC), Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Fundação Pro-Café (MAPA/PROCAFÉ) and Instituto Agronômico do Paraná (IAPAR), were used in the experiment.

The ten genotypes of *Coffea canephora* Pierre ex A. Froehner were developed by the breeding program of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), which is considered an international reference for breeding this species of coffee.

The group of genotypes was selected according to their characteristics of high crop yield and frequency of use in plantations of coffee in the southeast region of Brazil.

## 2.3. Determination of the Real Leaf Area

The leaves were collected from the middle section of the adult plants, along the third reproductive cycle (before harvest of 2013), on both sides of the cultivation row, being collected 10 mature and healthy leaves from the third and fourth pairs of each plant, counting from the apex of each branch.

Immediately after collection, the leaves were placed in humid plastic bags and taken to laboratory to determine the leaf area of each leaf separately.

The real leaf area was obtained using a leaf area integrator (area meter, LI 3100, LI-COR, precision: 0.01 $cm^2$), which uses grid cells of known area to determine the real area of the leaf that goes through the equipment [31].

## 2.4. Estimative of Leaf Area Using the Method of Linear Dimensions

The same collected leaves, now with known real area, were measured to determine the dimensions of their sheets. The largest dimensions were obtained by measuring the length and width of each leaf using rulers (precision: 0.1 cm).

The method of linear dimensions proposed by Barros *et al*. [16], for *Coffea arabica* L., is the most commonly used in Brazil. It estimates leaf area based on the dimensions of a rectangle limited by the leaf blade, adjusted by the equation: A = 0.667*(LW), where A is the leaf area and HW is the result of the multiplication of the greatest length (L) and the greatest width (W) of the leaf.

## 2.5. Data Analysis

The collected data were subjected to an analysis of variance using the F-test at 5% of probability in order to study the identify differences between genotypes and between methods to obtain the leaf area. The quadratic component of genotypic variance $\left(\hat{\phi}_g\right)$, the coefficient of genotypic determination ($H^2$), the coefficients of genetic variation ($CV_g$) and the variation index ($CV_g/CV_e$) were estimated based on the model $Y_{ijk} = \mu + G_i + A_j + GA_{ij} + \varepsilon_{ijk}$, considering the effect of genotypes as fixed. The means were studied using the Scott-Knott criteria at

5% of probability.

The Pearson's correlation coefficient was used to study the linear association of the values from the two different methods. The analyses were performed using the statistical software GENES [32] [33].

## 3. Results and Discussion

The overall mean for unitary leaf area for *C. arabica* was 47.33 $cm^2$ and for *C. canephora* was 47.08 $cm^2$, but the amplitude and variation for genotypes of *C. canephora* were higher, resulting in a coefficient of genetic variation 9.32% higher than for the group of genotypes of *C. arabica* (**Table 1**).

There was no significant effect for the method to estimate the leaf area, showing that the estimate values for leaf area were close enough of the real values to result in statistically similar means. The effect of the genotypic differences however was significant for both studies, demonstrating that the leaves of the genotypes presented some degree of morphologic differences. For both species, it was possible to notice greater effect of the difference between genotypes over the phenotypic variance than the effect of environmental factors, resulting in high estimate values for the coefficients of genotypic determination and variation indexes (**Table 1**).

Analyzing the leaf area obtained by the method of Barros *et al*. [16] and comparing it to the real leaf area, it is possible to notice the high level of similarity in the dispersion of the data (**Figure 1**), resulting in strong positive correlation between those variables.

**Table 1.** Mean squares (MS); means; estimatives of genotypic variance $(\hat{\phi}_g)$; coefficients of genotypic determination ($H^2$), coefficients of genetic variation ($CV_a$) and variation indexes ($CV_a/CV_e$) of the leaf area obtained with different methods in improved genotypes of *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner.

| Genotype | Coffee species | |
|---|---|---|
| | *Coffea arabica* L. | *Coffea canephora* Pierreex A. Froehner |
| $MS_{genotypes}$ | 2359.07** | 4638.16** |
| $MS_{methods}$ | 149.19ns | 567.84ns |
| $MS_{interaction}$ | 17.95ns | 47.45ns |
| Mean | 47.33 | 47.08 |
| $(\hat{\phi}_g)$ | 117.05 | 229.53 |
| $H^2$ | 99.23 | 98.97 |
| $CV_g$(%) | 22.85 | 32.17 |
| $CV_g/CV_e$ | 0.93 | 1.25 |

*Significant at 5% and **Significant at 1% of probability, by the F test.

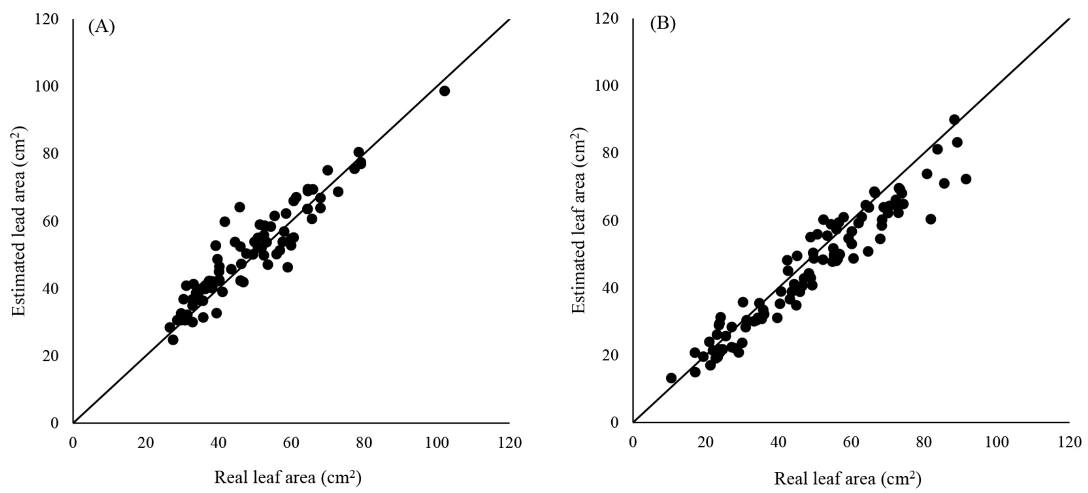


**Figure 1.** Dispersion between the real leaf area and the estimated leaf area by the method of linear dimensions (Barros), for leaves of improved genotypes of *Coffea arabica* L. (A) and *Coffea canephora* Pierre ex A. Froehner (B).

Differences between the leaf areas of the genotypes were observed for both *C. arabica* and *C. canephora*, being possible to discriminate three homogeneous groups regarding the size of the leaves for each species (**Table 2**). The group with larger leaves was formed by the genotypes Katipó, IAPAR 59 and Paraíso MG H419-1 for *C. Arabica*; and singly by the genotype 83 for *C. canephora*. The genotypes Pau-Brasil MG1, Catuaí Amarelo 24/137, Sacramento MG1 and Catuaí Vermelho IAC 144 have smaller leaves considering the species *C. arabica*, as well as the genotypes 02 and 32 for *C. canephora*. Tupi 81, Catiguá MG3 and Araponga MG1 formed an intermediate group regarding leaf area for *C. arabica*, while the genotypes 24, 67, 73, 76, 22 and 77 formed the intermediate group for *C. canephora*.

This different capacity to express leaf growth in a specific environment is an intrinsic characteristic of each improved genotype. The size of leaves is normally related to the plant adaptation to the environment and the development of larger leaf area can promote the photosynthetic efficiency. Since the photosynthetic rate is affected by leaf area and the way the radiation solar is intercepted, it is directly influenced by leaf size and plant architecture [34].

Other studies have reported wide genetic variability and different behavior between genotypes of coffee. Different patterns of growth and plant architecture are described for many improved genotypes, as well as different responses for modulated environments, which leaded to changes in the growth of the plant and development of leaf area [13] [21] [35] [36].

Strong and positive correlations between the leaf area estimated by the method of linear dimensions proposed by Barros *et al*. [16] and the real leaf area were observed for the improved genotypes of *C. arabica* and *C. canephora* (**Table 3**). For *C. arabica*, the genotype Tupi 81 presented the highest correlation (0.9571), while Katipó had the lowest (0.8972). For *C. canephora*, the genotype 73 showed the highest correlation (0.9928) and the genotype 153 had the lowest (0.8635).

Overall, the method proposed by Barros *et al*. [16] is efficient to estimate the leaf area of the improved genotypes of coffee, presenting very high correlations with the real leaf area, and its use is reliable for both species of coffee.

Studies made by Carvalho *et al*. [5], in order to compare plants of *C. arabica* and *C. canephora* regarding the photosynthetic rates and aspects of leaf anatomy, concluded that the basic shape and positioning of leaves are similar for both species of coffee, being described as opposite, crossed, with short petiole, blades with elliptic to elliptic-lanceolate shape and wavy margins. Despite the differences in size, thickness, coloration, among other leaf traits among species of coffee, the pattern of shape for the leaves is similar, which explains the good fit of the model proposed by Barros *et al*. (1973) even for the species *C. canephora* to estimate leaf area.

Even with the differences in leaf size among genotypes, the leaf shape remained similar, therefore, even if the cultivation conditions allow the genotypes to show different growth rate, the linear dimensions of the leaf blade keep being good variables to estimate its area and the model proposed by Barros *et al*. [16], maintaining good

**Table 2.** Means of leaf area of improved genotypes of *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner.

| *Coffea arabica* L. | | *Coffea canephora* Pierre ex A. Froehner. | |
|---|---|---|---|
| Genotype | Leaf area ($cm^2$) | Genotype | Leaf area ($cm^2$) |
| Katipó | 60.85 a | 83 | 71.21 a |
| IAPAR 59 | 58.88 a | 24 | 59.82 b |
| Paraíso MG H419-1 | 58.08 a | 67 | 57.04 b |
| Tupi 81 | 52.51 b | 73 | 53.56 b |
| Catiguá MG3 | 50.57 b | 76 | 49.13 b |
| Araponga MG1 | 46.63 b | 22 | 48.77 b |
| Pau-Brasil MG1 | 41.55 c | 77 | 48.34 b |
| Catuaí Amarelo 24/137 | 41.24 c | 02 | 33.93 c |
| Sacramento MG1 | 34.48 c | 32 | 25.26 c |
| Catuaí Vermelho IAC 144 | 28.45 c | 153 | 23.77 c |

Means followed by the same letter in each column do not differ by the Scott-Knott test at 5% of probability.

**Table 3.** Correlations between the real leaf area and the estimated leaf area by the method of linear dimensions (Barros), for leaves of improved genotypes of *Coffea arabica* L. and *Coffea canephora* Pierre ex A. Froehner.

| *Coffea arabica* L. | | *Coffea canephora* Pierreex A. Froehner. | |
|---|---|---|---|
| Genotype | Correlation | Genotype | Correlation |
| Tupi 81 | 0.9571 | 73 | 0.9928 |
| Catuaí Vermelho IAC 144 | 0.9417 | 77 | 0.9877 |
| Catiguá MG3 | 0.9406 | 76 | 0.9557 |
| IAPAR 59 | 0.9348 | 32 | 0.9128 |
| Catuaí Amarelo 24/137 | 0.9271 | 24 | 0.9055 |
| Araponga MG1 | 0.9226 | 02 | 0.8915 |
| Paraíso MG H419-1 | 0.9135 | 22 | 0.8906 |
| Sacramento MG1 | 0.9103 | 83 | 0.8896 |
| Pau-Brasil MG1 | 0.9054 | 67 | 0.8752 |
| Katipó | 0.8972 | 153 | 0.8635 |

accuracy to describe it. These similarities in shape of the leaves were also observed in other studies involving species of coffee [24], which observed that power models based on the combination of length and width of the leaves presented desirable fit to estimate leaf area for eight coffee genotypes.

## 4. Conclusions

The genotypes for both *Coffea arabica* L. and *Coffe acanephora* Pierre ex A. Froehner have different leaf areas, making it possible to discriminate groups of genotypes for both species using the size of their leaves as classification criteria.

Even with the differences between genotypes, the pattern of leaf shape remains similar, confirming the applicability of the method to estimate leaf area using linear dimensions of leaves for improved genotypes, such as the model proposed by Barros, without the occurrence of loss of accuracy for the improved genotypes, for both Arabica and Conilon coffee, studied in this experiment.

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