Digital imaging of coffee leaves under different nitrogen concentrations applied to soil

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ABSTRACT

Brazil is the largest *Coffea arabica* L. producer and exporter in the world market. In view of the need for nitrogen fertilization, the objective of this work was to evaluate the use of digital images of coffee leaves as a function of nitrogen concentration and cultivars, as well as to evaluate the most adequate sampling of this technique to predict leaf nitrogen. The experiment was set in a greenhouse, at UNESP - Experimental Campus of Registro-SP, using 12L pots with medium sand. A completely randomized design was adopted, in a 4 x 2 factorial scheme, with four concentrations of N (0, 50, 100 and 200 mg dm^{-3}), and two coffee cultivars (Mundo Novo and Obatã), with ten replications. The variables evaluated were: hue, dark green color index (DGCI), leaf area and leaf nitrogen content. The hue and DGCI were influenced by N concentration, cultivar and are dependent on the day after treatment application and the number of leaves used. The use of five leaves per replication was more adequate to estimate leaf N content. Hue, DGCI and leaf area were higher in Obatã depending on the day after application of the treatment and N concentration.

Keywords: hue; dark green color index; *Coffea arabica*; leaf nitrogen.

INTRODUCTION

Brazil is the largest *Coffea arabica* L. producer and exporter in the world market, with an yield around 63.08 million bags of Arabica coffee in 2020 (Conab, 2020). In this context, nitrogen (N) is a macronutrient of fundamental importance, for being one of the elements most required by plants, due to its importance in the production of new cells and tissues (Santos *et al.*, 2016). The content of this nutrient directly interferes with the growth, development and production of crops (Silva *et al.*, 2018).

The low availability of N significantly alters the assimilation capacity of CO_2, affecting the production of photoassimilates and the growth rate as a whole, compromising the structural characteristics of the plants (Gimenes *et al.*, 2017). Excess of nitrogen in the soil is also harmful for plants due to the imbalance and antagonism caused to other nutrients (Fagundes, 2011). The correct concentration of nutrients is essential to minimize losses and achieve good results for the crop. Fertilization with N has high mobility in the soil, requiring frequent monitoring of its availability to plants (Oliveira *et al.*, 2020).

The monitoring of the crop and the diagnosis of N in the field mobile devices that estimate chlorophylls *a* and *b* and others devices that simultaneously capture the image and establish the RGB color indices, generating an estimate of leaf N, provide promising results (Wang *et al.*, 2014). Show an association of chlorophyll with available nitrogen contents (Santos & Castilho, 2015), this correlation being

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associated with the hue and green color of the leaves.

In this context, digital image processing is a plausible technological option, capable of predicting the nutritional status of N in a simple and fast way, in comparison with laboratory analyses, based on the parameters of hue and dark green color index (DGCI) (Felisberto *et al.*, 2016). Given the above, the objective of this work was to evaluate the use of digital images of coffee leaves as a function of nitrogen concentration and different cultivars, as well as to evaluate the most adequate sampling of this technique to predict leaf nitrogen.

MATERIAL AND METHODS

The experiment was set on January 5, 2015, in a greenhouse, at the Experimental Campus of Universidade Estadual Paulista, located in the city of Registro-SP, Southeastern Brazil (24º29'15" S, 47º50'37" W), with an average altitude of 25 m.

The experiment was carried out in a completely randomized design, in a 4 x 2 factorial scheme with four concentrations of nitrogen (0, 50, 100 and 200 mg dm^{-3}) and two coffee (*Coffea arabica* L.) cultivars (Mundo Novo and Obatã), with ten replicates for each treatment, totaling 80 plots. Each experimental plot contained a coffee seedling. Urea was used as a source of N, applied in coverage, divided into four plots, with an interval of 30 days between applications; the first was carried out on January 20, 2015. River sand was used as a substrate to conduct the experiment, and corrections were then made. Base saturation was increased to 50% using dolomitic limestone (65% PRNT) and the levels of P and K, for 200 and 100 mg dm^{-3}, using simple superphosphate and potassium chloride, respectively.

Coffee seedlings produced in bags were transplanted to containers measuring 30 x 20 x 26 cm; upper, lower diameter and height, respectively, with 12 L of sand, where the treatments were applied. The pots were arranged on top of pallets, with spacing of 30 cm between plants and of approximately 80 cm between rows. Irrigation was by micro sprinklers and the ideal amount of water to conduct the experiment was based on the field capacity (FC) of the substrate, adopting 70% FC, reaching a value of 350 ml a day.

Digital images were obtained on the 60 and 90 DAA, from leaf newly expanded on valuation date. They were obtained with the use of a digital camera (Cybershot DSC-H200 Sony 20. Megapixels, Japan), with a resolution of 20 MP, without the flash, using the macro function. The image background was pink. The distance between the camera and the sheet was also standardized at 20 cm and the photos were taken from 9:00 am to 11:00 am. The images were read by RGB analysis, with the aid of Corel Photo Paint® (Corel Draw Graphics Suite X4, COREL Corporation, Canada). In the case of an image that has information in different frequency intervals, an f (x, y) function is required for each band. This is the case of standard RGB color images, which are formed by the information of primary additive colors, such as red (R - Red), green (G - Green) and blue (B - Blue) (Filho & Neto 1999). Calculations defining by the RGB hue terms: (Paulidis & Liow, 1990) and dark green colors index (Wang *et al.*, 2013).

In the last evaluation, 90 DAA of the treatment, the leaves from which the digital images were obtained, were collected, dried in a circulation oven air renewal for drying at 65 °C, for 72 hours, to determine the dry phytomass of the leaves, ground and sent to the laboratory for the determination of N content, from acid digestion and distillation (Kjeldahl) according to the methodology of Malavolta *et al.* (1997). Concerning the RGB, the green and circular cards used as color standards, known areas; therefore, with the aid of reading the pixels of the leaves and, it was possible to determine the leaf area. In the last leaf collection, a photo of one leaf was taken by replication (ten per treatment) and, subsequently, of a sample of five leaves per replication (fifty per treatment), in order to compare which is the most appropriate methodology. The images were all cropped in Windows Paint® (Microsoft, EUA) and then, with the help of Corel Photo Paint®, in the mask function, later on, color mask, it was possible to redefine the color of the leaves. The amount of RGB of each leaf was read in the image function and, later, a histogram of the same tool, in other words, the histogram of the distribution of RGB values of the image of the leaves is a tool of Corel Photo Paint and, through this tool, it is obtained the average value of R, G and B of the image.

The green and yellow cards, according to the literature, are of known colors, thus enabling a correction factor and standardizing the photos, regardless of brightness or time of the sections (Rorie *et al.*, 2011). The reference colors were selected in Corel Draw® and the cards were printed and cut out.

The results were submitted to analysis of variance, regression adjustment by linear and quadratic models, and linear correlation using the Sisvar v program. 5.3. (Ferreira, 2011). The average values were compared by the test Tukey to 5% of significance. For the correlation, it was considered as variable x the content of leaf N and as variable y, the characteristics evaluated in the leaf image (hue, DGCI and leaf area) of one leaf or five leaves per repetition. As for the determination of the leaf N content, the samples of the repetitions were grouped two by two, for the correlation the other indexes were also grouped two by two, totaling 40 data for each variable.

RESULTS AND DISCUSSION

First evaluation date (60 days after application of the treatment – DAA)

For hue, there was an effect only for N concentrations. There was no significant interaction between the variation factors. Both cultivars showed equal responses to nitrogen concentrations (Table 1). The hue of the leaf on the cultivar Mundo Novo, plants showed a linear increase in relation to N concentrations, where the highest index was found at 200 mg dm^{-3} (116 degrees), while the hue of Obatã shows a quadratic adjustment; the maximum point can be established, presenting the highest hue value (113 degrees) at 179 mg dm^{-3} N. For the DGCI, there was an effect only for N concentrations. There was no significant interaction between factors. Both cultivars showed equal responses to nitrogen concentrations (Table 2). Both Mundo Novo and Obatã showed linear growth, where the highest DGCI observed was at 200 mg dm^{-3} N. The behavior of linear regression is an indication that the increase in N concentrations improves the level of the DGCI in the cultivars. Hue and DGCI had similar behavior, with no distinction between cultivars and differences in N concentrations, in relation to the evaluation time of 60. That the application of concentrations of N influence the green color of leaves, due to the increase in the of chlorophyll, the higher concentration of N in tissues (Groff *et al.*, 2020). Which implies predicting the correlation of hue and DGCI with leaf N availability.

As for the leaf area, there was an effect for coffee cultivars, and Obatã showed an average leaf area 10 cm^2 higher than Mundo Novo. There was no significant interaction between factors (Table 3). The coefficient of variation of this characteristic was high due to the evaluation of a single leaf per replication. The cultivar Obatã showed a linear increase in leaf area in relation to N concentrations, where the largest average was 62.26 cm^2 obtained at 200 mg dm^{-3}, while for Mundo Novo, there was no adjustment, neither linear nor quadratic, with an average of 42.67 cm^2.

According to Leghari *et al.* (2016), the increase in leaf area as a function of “soil N” was already expected, as it is a mineral nutrient that is more limiting to plant growth, as it is an integral part of molecules that are fundamental to metabolism. However, as seen in this research (Table 3), the response to nutrient uptake efficiency N can be variable for coffee cultivars.

Second evaluation date (90 days after application of the treatment – DAA)

For hue, there was an effect for cultivar and N concentrations. There was no significant interaction. The two cultivars showed different responses to nitrogen concentrations (Table 4). There was a difference in hue between the two cultivars in the treatments using 50 and 100 mg dm^{-3}, and Obatã showed higher hue values (Table 4). According to Felisberto *et al.* (2016), the hue of the leaf digital image was significantly influenced by N concentrations, ranging from 75.1 to 149.7 degrees in studies 51, 79, 93, 107, 121 and 149 days after transplantation, at 5; 10; 15; 20 and 25g of N for the pepper plant. The authors also mention that the lowest hue values corresponded to plants with N deficiency.

The hue for leaves of Mundo Novo plants showed a linear increase in relation to N concentrations, where the highest hue index was found at 200 mg dm^{-3} (97 degrees), while the hue value of Obatã demonstrated a quadratic adjustment, presenting the highest value (99.37 degrees) for hue at 100 mg dm^{-3} N. The behavior of the cultivars for the hue parameter were similar for the leaf N content (Table 10). In this sense, the difference in concentrations of N provides different shades of green in the leaf. The difference in cultivar performance and the correlation with the of leaf N is evident. For DGCI, there was an effect for cultivar and N concentrations. There was no significant interaction. The two cultivars did not show equal responses for nitrogen concentrations (Table 5). There was a difference in the DGCI between the two cultivars, at all N concentrations, being higher in Obatã. The DGCI in the two cultivars showed a quadratic adjustment, as a function of N concentrations. The cultivar Obatã obtained a maximum DGCI point with a lower N concentration (150 mg dm^{-3} N) in relation to Mundo Novo (200 mg dm^{-3} N). Similarly, in the study by Bertani *et al.* (2019), the DGCI in relation to N concentrations also obtained a quadratic adjustment, with a maximum point of (680 mg dm^{-3} N) in

Table 1: Summary of analysis of variance, regression analysis and comparison of hue averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Sixty days after application of the treatments (DAA). Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.5192		
Doses (D)	< 0.001		
C X D	0.8032		
CV, %	16.22		
	Hue (degrees)		
Mundo Novo	94.98 a		
Obatã	97.24 a		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	75.1 A	79.1 A	77.1
50	80.8 A	83.9 A	82.36
100	108.5 A	113.9 A	111.2
200	115.6 A	112.5 A	113.8
		Pr > t	
Regression	0.001 L[1]	0.033 Q[2]	0.013 Q[3]
R^2	86.1	83.1	87.9
[1] $\hat{y} = 0.2176x + 75.96$	$^3\hat{y} = -0.001x^2 + 0.407x + 73.67$		
[2] $\hat{y} = -0.0012x^2 + 0.429x + 75.36$			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Table 2: Summary of analysis of variance, regression analysis and comparison of dark green colour index averages (DGCI), as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Sixty days after application of the treatments (DAA). Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.3523		
Doses (D)	< 0.001		
C X D	0.7638		
CV, %	22.86		
	Dark green color index		
Mundo Novo	0.53 a		
Obatã	0.55 a		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	0.357 A	0.419 A	0.388
50	0.397 A	0.428 A	0.412
100	0.648 A	0.680 A	0.664
200	0.719 A	0.698 A	0.708
		Pr > t	
Regression	< 0.001 L[1]	< 0.001 L[2]	0.0449 L[3]
R^2	76.13	86.35	85.79
$^1\hat{y} = 0.0020x + 0.3588$	$^3\hat{y} = 0.0018x + 0.3888$		
$^2\hat{y} = 0.0016x + 0.419$			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Table 3: Summary of analysis of variance, regression analysis and comparison of leaf area averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Sixty days after application of the treatments (DAA). Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.0375		
Doses (D)	0.2186		
C X D	0.1169		
CV, %	43.05		
	Leaf area (cm²)		
Mundo Novo	42.67 a		
Obatã	52.36 b		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	45.83 A	41.27 A	43.550
50	40.82 A	43.61 A	42.244
100	46.10 A	62.34 A	54.218
200	37.87 A	62.26 A	50.069
		Pr > t	
Regression	ns	0.0098 L[1]	ns
R²	ns	74.09	ns
	$^1\hat{y} = 0.116x + 42.222$		

Means followed by the same letter do not differ by the Tukey test at 5% significance. ns – not significant; L –linear; Q – quadratic.

Table 4: Summary of analysis of variance, regression analysis and comparison of hue averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of one leaves per replication. Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.0006		
Doses (D)	< 0.001		
C X D	0.1568		
CV, %	7.17		
	Hue (degrees)		
Mundo Novo	87.77 a		
Obatã	92.96 b		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	78.38 A	80.1 A	79.24
50	85.52 A	93.44 B	89.48
100	90.26 A	99.37 B	94.82
200	96.94 A	98.95 A	97.95
		Pr > t	
Regression	< 0.001 L[1]	< 0.001 Q[2]	< 0.001 Q[3]
R²	96.56	99.62	99.77
$^1\hat{y} = 0.09x + 79.9$		$^3\hat{y} = -0.0007x^2 + 0.2232x + 79.435$	
$^2\hat{y} = 0.001x^2 + 0.2984x + 80.371$			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Table 5: Summary of analysis of variance, regression analysis and comparison of dark green color index averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of one leaves per replication Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.0012		
Doses (D)	< 0.001		
C X D	0.5482		
CV, %	12.49		
	Dark green color index		
Mundo Novo	0.509 a		
Obatã	0.560 b		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	0.406 A	0.433 A	0.420
50	0.489 A	0.556 B	0.523
100	0.554 A	0.631 B	0.593
200	0.590 A	0.621 B	0.606
		Pr > t	
Regression	0.0260 Q[1]	< 0.001 Q[2]	< 0.001 Q[3]
R²	99.23	99.99	99.97

[1]$\hat{y} = -0.000005x^2 + 0.00201x + 0.4049$ [3]$\hat{y} = -0.000008x^2 + 0.00251x + 0.4192$

[2]$\hat{y} = -0.000010x^2 + 0.00301x + 0.4325$

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

passion fruit seedlings. For leaf area, there was an effect for cultivar and N concentrations. There was no significant interaction. Both cultivars showed quadratic adjustment in terms of leaf area as a function of N concentrations, with a very similar maximum point, differing only at 50 mg dm^{-3} N (Table 6). There was a difference in leaf area between the two cultivars, and the average leaf area of Obatã (46 cm^2), was greater than that of Mundo Novo (39.02 cm^2), as verified in the first evaluation. In this study, the cultivar Obatã was the most responsive (Table 6) to nitrogen concentrations and the leaf area followed the quadratic behavior of the leaf N content (Table 10), that is, greater leaf area resulting from higher leaf N content, which is favored by the availability of N in the soil. In the study of Schwers *et al.* (2016), they also verified the difference in leaf area in response to different plant cultivars as a function of nitrogen fertilizer doses.

Data obtained from a sample consisting of five leaves 90 days after application of the treatment – DAA)

For hue, there was an effect for cultivar and N concentration. There was no significant interaction. The cultivars showed different responses to nitrogen concentration (Table 7). There was a difference in hue between the two cultivars at concentrations of 50 and 100 mg dm^{-3}; Obatã stands out in relation to Mundo Novo one more time. The hue of the leaf from Mundo Novo plants showed a linear increase in relation to N concentrations, where the highest index was found at 200 mg dm^{-3} N (108 degrees), while the Obatã demonstrated quadratic adjustment, presenting the highest value (113 degrees) at 153 mg dm^{-3} N. The hue of the leaves mainly reflects the possible differences in the contents of leaf N, and, consequently, in the concentrations of chlorophylls, which may influence the photosynthetic capacity of the plant (Flexas *et al.*, 2016). In this context, the hue and DGCI obtained from the digital image have shown to be promising, as they present high relationships with nitrogen concentrations and, consequently, an indicator of available leaf nitrogen.

For DGCI, there was an effect for cultivar and N concentrations. There was no significant interaction. The two cultivars did not show equal responses for nitrogen concentrations (Table 8). There was a difference in the DGCI between the two cultivars at 50 and 100 mg dm^{-3} N. The intensity of the leaf green

Table 6: Summary of analysis of variance, regression analysis and comparison of leaf area averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of one leaves per replication. Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.0207		
Doses (D)	< 0.001		
C X D	0.5941		
CV, %	31.83		
	Leaf area (cm²)		
Mundo Novo	39.02 a		
Obatã	46.19 b		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	21.53 A	27.15 A	24.34
50	32.25 A	46.39 B	39.34
100	50.31 A	53.33 A	51.86
200	51.89 A	57.90 A	54.90
		Pr > t	
Regression	0.0249 Q[1]	0.0150 Q[2]	0.0012 Q[3]
R²	95.43	98.74	99.66
	[1]$\hat{y} = -0.0011x^2 + 0.3827x + 19.9874$	[3]$\hat{y} = -0.0012x^2 + 0.3870x + 23.9429$	
	[2]$\hat{y} = -0.0012x^2 + 0.3899x + 27.9024$		

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Table 7: Summary of analysis of variance, regression analysis and comparison of hue averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}) in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of five leaves per replication. Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.0003		
Doses (D)	< 0.001		
C X D	0.1227		
CV, %	7.81		
	Hue (degrees)		
Mundo Novo	93.01 a		
Obatã	99.36 b		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	78.18 A	80.73 A	79.46
50	87.18 A	96.78 B	91.99
100	98.90 A	110.14 B	104.52
200	107.77 A	109.80 A	108.72
		Pr > t	
Regression	0.001 L[1]	< 0.001 Q[2]	< 0.001 Q[3]
R²	95.36	99.43	99.25
[1]$\hat{y} = 0.14859x + 80.006$		[3]$\hat{y} = -0.000948x^2 + 0.3397x + 78.8911$	
[2]$\hat{y} = -0.00140x^2 + 0.4290x + 80.2120$			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

presented by the cultivars, is an important value to estimate the indices of photosynthetic pigments (Conceição *et al.*, 2019), so that changes in the photosynthetic process may have consequences on coffee yield. The two cultivars demonstrate quadratic adjustment, where the maximum point of Mundo Novo was 200 mg dm^{-3} N, with DGCI of 0.677, and that of Obatã was 100 mg dm^{-3} N, to obtain the DGCI 0.690.

For leaf area, there was an effect for cultivar and N concentrations. There was no significant interaction. Both cultivars showed equal responses to N concentrations (Table 9). There was a difference in leaf area averages between cultivars Mundo Novo and Obatã; the latter showed the largest leaf area, with a gain of 10.65%. The leaf area of Mundo Novo and Obatã showed a quadratic adjustment in relation to N concentrations. In the study of Colodetti *et al.* (2015), the leaf area of the coffee tree also increased with the concentrations of N, which compromises the efficiency of the photosynthetic rate of the plant, as well as its yield.

For N content in the coffee leaf, there was an effect only for N concentrations (Table 10). Bertani *et al.* (2019), stated that the N content was influenced in the leaves of passion fruit seedlings, that is, leaf N content increased with the increment in nitrogen fertilization represented by the quadratic model. The dose 0.34 g L^{-1} had the lowest content, with an average of 21 g kg^{-1}, and can be indicated, considering that it reached the greatest plant development, the highest DGCI and leaf area, very close at the maximum dose of 0.68 g L^{-1}. This result corroborates thosse found in this experiment for hue and leaf N content, in which the maximum value for Mundo Novo, was found at the highest N concentration, while for Obatã, the maximum values of hue and DGCI were obtained lower N concentration.

Regarding regression, leaf N content of cultivar Mundo Novo showed linear development in response to N concentrations, where the point of maximum response was in relation to 200 mg dm^{-3} of 25.2 g kg^{-1}. In Obatã, there was a quadratic adjustment; the maximum point was the concentration 160 mg dm^{-3}, with a content of 26 g kg^{-1}. These data corroborate those of Teixeira *et al.* (2020), a quadratic adjustment was observed with increasing N concentration, inducing a greater accumulation of leaf N in the lemon tree.

Table 8: Summary of analysis of variance, regression analysis and comparison of dark green color index averages (DGCI), as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of five leaves per replication. Registro, SP, Brazil.

FV		**Pr > Fc**	
Cultivars (C)		< 0.001	
Doses (D)		< 0.001	
C X D		0.1259	
CV, %		10.68	
		Dark green color index	
Mundo Novo		0.534 a	
Obatã		0.593 b	
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	0.396 A	0.430 A	0.413
50	0.483 A	0.567 B	0.525
100	0.592 A	0.690 B	0.641
200	0.667 A	0.687 A	0.709
		Pr > t	
Regression	0.0203 Q[1]	< 0.001 Q[2]	< 0.001 Q[3]
R²	99.07	99.14	99.09
[1] $\hat{y} = -0.000005x^2 + 0.00241x + 0.3903$		[3] $\hat{y} = -0.000007x^2 + 0.00295x + 0.4082$	
[2] $\hat{y} = -0.000012x^2 + 0.00376x + 0.4243$			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Table 9: Summary of analysis of variance, regression analysis and comparison of leaf area averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}) in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of five leaves per replication. Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.0496		
Doses (D)	< 0.001		
C X D	0.1498		
CV, %	25.25		
	Leaf area (cm²)		
Mundo Novo	34.95 a		
Obatã	39.12 b		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	15.49 A	20.86 A	18.17
50	30.47 A	38.62 A	34.55
100	43.09 A	50.39 A	46.74
200	50.74 A	46.61A	48.67
		Pr > t	
Regression	0.0057 Q[1]	< 0.001 Q[2]	< 0.001 Q[3]
R²	99.86	99.85	99.85
[1]ŷ = -0.00095x2 + 0.3681x + 15.203	[3]ŷ = -0.00129x2 + 0.4117x + 17.9005		
[2]ŷ = -0.00162x2 + 0.4551x + 20.605			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Table 10: Summary of analysis of variance, regression analysis and comparison of leaf N averages, as a function of nitrogen concentrations (0, 50, 100 and 200 mg dm^{-3}), in Mundo Novo and Obatã coffee seedlings. Ninety days after application of the treatments (DAA)-Sample of five leaves per replication. Registro, SP, Brazil.

FV	**Pr > Fc**		
Cultivars (C)	0.2459		
Doses (D)	< 0.001		
C X D	0.0987		
CV, %	9.03		
	Leaf N content (g kg^{-1})		
Mundo Novo	21.85 a		
Obatã	22.60 a		
Doses (mg dm^{-3})	**Mundo Novo**	**Obatã**	**Mean**
0	18.4 A	18.6 A	18.5
50	20.2 A	21.0 A	20.7
100	22.4 A	25.8 B	24.1
200	25.2 A	26.0 A	25.6
		Pr > t	
Regression	< 0.001 L[1]	0.0157 Q[2]	0.0282 Q[3]
R²	97.39	89.91	97.69
[1]ŷ = 0.034171x + 18.56	[3]ŷ = -0.000165x2 + 0.0700x + 18.2572		
[2]ŷ = -0.000275x2 + 0.0949x + 18.1472			

Means followed by the same letter do not differ by the Tukey test at 5% significance. L – linear; Q – quadratic.

Pearson correlation between the parameters evaluated

There was a significant and positive correlation of the nitrogen content in the leaf with hue (H), DGCI and leaf area (AF), with the exception only for AF, 60 DAA (Table 11). The highest correlation coefficients between leaf N content and hue, DGCI and leaf area were obtained when five leaves were used per replication. The highest correlation coefficient with leaf N content was obtained with DGCI, 90 DAA, when five leaves were used per replication in the analysis. In general, the correlations of hue, DGCI and leaf area were associated with N concentrations and influenced by leaf N content. The results corroborate Gazola *et al.* (2016), this is, emphasize the efficiency of the use of digital imaging in recommending nitrogen fertilization.

Table 11: Summary of data correlation between nitrogen content and hue, dark green color index and leaf area in Mundo Novo and Obatã coffee seedlings at different collection times, 60 and 90 days after the application of treatments. Registro, SP, Brazil.

Leaf N content	One leaf/ replication, 60 DAA	One leaf / replication, 90 DAA	Five leaves / replication, 90 DAA
Hue	0.53***	0.71***	0.75***
Dark green color index	0.57***	0.71***	0.76***
Leaf area	0.30 ns	0.52***	0.59***

*, ***: significant by t-test at 5 and 0.1% probability; ns- not significant.

CONCLUSIONS

The hue and DGCI obtained in the digital image analysis of the coffee leaf were influenced by N concentrations, cultivar and are dependent on the day after treatment application and the number of leaves used.

The use of five leaves per replication in digital image analysis was more adequate to estimate leaf N content.

Hue, DGCI and leaf area were higher in cultivar Obatã compared to Mundo Novo, and are dependent on the day after application of the treatment and N concentrations.

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