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Spectral indexes for identification of nitrogen deficiency in maize[1]

Índices espectrais para identificação de deficiência de nitrogênio em plantas de milho

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ABSTRACT - Image analysis can provide information extracted from the leaves of crops, and contribute to early identification of nutrient deficiency. The objective of this study was to recognize nutritional nitrogen (N) patterns in maize plants, at the V4 and V7 stages, using digital image analysis based on spectral indexes. The experiment was carried out in a greenhouse under hydroponic cultivation. Treatments consisted of a completely randomized design, in a 4 × 2 factorial arrangement, with four replications. The factors were constituted by the doses of N (0; 3.0; 6.0 e 15 mmol L^{-1}) combined at V4 and V7. In each stage, digital images were taken of leaf blades, with subsequent chemical composition and image analysis. For image recognition and classification, a vector of characteristics based on the spectral indexes was used as follows: excess of green, normalized red, normalized green and red-green ratio, and the combination among them. Additionally, extracted blocks of 9 × 9, 20 × 20 and 40 × 40 pixels on original images were used. The N content in the leaf blade, the dry mass of the plants and the external critical level of N in the nutrient solution were determined for result validation, based on 90% dry matter production. Maximum the global accuracy rate for N patterns was 80 and 93% at V4 and V7, respectively. The use of combined spectral indexes provided better classification performance, and the 9 × 9 pixel image block appeared more adequate for differentiation among the doses of N.

Key words: *Zea mays* L.. Image analysis. Mineral nutrition. Image processing.

RESUMO - A análise de imagens pode fornecer informações extraídas das folhas das culturas, que podem contribuir na identificação precoce de deficiência de nutrientes. O objetivo do trabalho foi reconhecer padrões nutricionais de nitrogênio (N) em plantas de milho, nos estádios V4 e V7, utilizando análise de imagens digitais baseados em índices espectrais. O experimento foi realizado em casa de vegetação sob cultivo hidropônico. Os tratamentos foram dispostos em um delineamento inteiramente casualizado, em esquema fatorial 4×2, com quatro repetições. Os fatores foram constituídos pelas doses de N (0; 3,0; 6,0 e 15 mmol L^{-1}) combinados no V4 e V7. Em cada estádio foram digitalizadas imagens da lâmina foliar, que foram processadas pela análise de imagens e analisadas quimicamente. Para reconhecimento das imagens foi utilizado um vetor de características baseados nos índices espectrais: excesso de verde, vermelho normalizado, verde normalizado e razão verde-vermelho, e na combinação entre eles. Na imagem original foram extraídos blocos de 9×9; 20×20 e 40×40 pixels. Para validação dos resultados foi determinado o teor de N na lâmina foliar, a massa seca das plantas e o nível crítico externo de N na solução nutritiva, com base em 90% produção de massa seca. A máxima porcentagem de acerto global dos padrões de N foi 80 e 93% em V4 e V7, respectivamente. A utilização de índices espectrais combinados proporcionou melhor desempenho na classificação, e o bloco de imagem 20×20 pixels mostrou-se mais adequado para a diferenciação entre as doses de N.

Palavras-chave: *Zea mays* L.. Análise de imagem. Nutrição mineral. Processamento de imagens.

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INTRODUCTION

Maize is one of the most nutrient-demanding crops, with abundant nitrogen (N) a requirement for growth. According to Von Pinho *et al.* (2009), maize plants accumulate 22% of the total N at the V3 stage, reach 60% at V6, and extract and estimated 374.6 kg ha^{-1} at V10. Thus, determining what period the crops most require nutrients allows correcting deficiencies that may occur during development, as well as correctly estimating the amount of necessary fertilizer, and stablishing management practices, in order to maximize utilization. The need for efficiency in nutrient usage and improvement of methods to identify the plant nutritional state has led Agronomy, Economy, Environment and Operational Systems to search for new technologies (ROMUALDO *et al.*, 2014).

Precision agriculture has used new technologies as a tool to assist in nutritional diagnoses and high productivity plant management. As an example, the use of optical sensors to capture the reflectance at certain wavelengths throughout the plant canopy, for applying N at a variable rate (AMARAL; MOLIN, 2011). Additionally, systems of computational vision through image analysis (CAMARGO; SMITH, 2009; PEDROSO *et al.*, 2010), and the most recent studies using unmanned aerial vehicles for image acquisition (HOLLAND; SCHEPERS, 2010, 2012; SHIRATSUCHI *et al.*, 2011) have been developed. Mathematical combinations of different spectral bands within their electromagnetic spectrum (ATZBERGER, 2013) can better evaluate the reflectance characteristic usage in agriculture. These combinations are measures for a particular purpose, within the bands of the visible and near infrared (SHIRATSUCHI *et al.*, 2011).

Image analysis uses methods to interpret and/or verify an image automatically or semi-automatically (BACKES; CASANOVA; BRUNO, 2009). Image analysis is a promising tool for the early diagnosis of nutritional maize status, detecting levels of latent nutritional deficiencies that can allow for the improvement of nutrient management in the crop cycle. This technology has the potential to recommend nitrogen topdressing in maize submitted to different management systems (DELLINGER; SCHIMDT; BEEGLE, 2008), and for maize nutritional diagnosis in the greenhouse or in the field (ROMUALDO *et al.*, 2014; SILVA *et al.*, 2014). Moreover, it may be used for size processing, width, and length analysis of corn seeds (TEIXEIRA *et al.*, 2007).

Thus, the objective of this research was to recognize patterns for nitrogen nutritional deficiencies in maize plants, at the V4 and V7 developmental stages, using digital image analysis of leaf blades based on visible spectral indexes.

MATERIAL AND METHODS

This study consisted of an image bank obtained from an experiment with the DKB 390® maize hybrid cultivated in a hydroponic system, and conducted in the agricultural science sector of the Department of Animal Science of the Faculty of Animal Science and Food Engineering - FZEA / USP, in Brazil.

A completely randomized experimental design was created, in a 4 × 2 factorial arrangement, with four replicates and factors composed of four doses of N concentration. For T1 = complete omission, T2 = 3.0 mmol L^{-1} (dose at 20% – 42 mg L^{-1}); T3 = 6.0 mmol L^{-1} (dose at 40% – 84 mg L^{-1}); T4 = 15.0 mmol L^{-1} (dose at 100% – 210 mg L^{-1}), evaluated in two phenological stages, V4 and V7 [24 and 44 days after emergence (DAE) respectively], totaling 32 experimental units.

Studies on nutrient solutions and on macronutrients followed Hoagland and Arnon (1950, respectively, and for micronutrients, Furlani *et al*. (1999), modified for preliminary tests. The nutrient solution had the following concentrations in L^{-1}: N = 210 (complete dose); P = 31; K = 234; Ca = 200; Mg = 48; S = 64; B = 0.6; Cu = 0.05; Fe = 6.9; Mn = 0.8; Mo = 0.032 and Zn = 0.17. Nutrients were provided according to reagent sources P.A: amonium nitrate (NH_4NO_3); potassium dihydrogen phosphate (KH_2PO_4); potassium chloride (KCl); calcium chloride dehydrate ($CaCl_2.2H_2O$); magnesium sulfate heptahydrate ($MgSO_4.7H_2O$); boric acid (H_3BO_3); copper chloride ($CuCl_2$); ethylenediamine di-2-hydroxyphenyl acetate ferric (Fe-EDDHA 6% - Fe); manganese chlorid tetrahydrate ($MnCl_2.4H_2O$); molybdic acid monohydrate ($H_2MoO_4.H_2O$) and zinc chlorid ($ZnCl_2$). The nutrient solution was prepared with deionized water, and the reagents were previously prepared in individual stock solutions, and pipetted into the concentrations.

Each experimental unit received filtered and bubbled compressed air for 10 seconds every 30 seconds, had nutrient solutions renewed weekly, had deionized water added when necessary, and had pH monitored daily and corrected occasionally with the use of 1N HCl (hydrochloric acid) and / or 1N NaOH (sodium hydroxide) to between 5.0 and 6.0 during the experimental period.

After sowing plants in washed sand, and irrigating the seedlings with deionized water, they were maintained under these conditions for 14 days after emergence (VE). Two plants composed each experiment, in 3.5 L of nutrient solution and were previously selected by uniformity and transplanted into the experimental units.

The leaves for digital images were harvested at 24 and 44 days after plant emergence (DAE), at the V4 and V7 developmental stages, respectively. The indicative leaf blades of each phenological stage were extracted from the fourth fully expanded leaf at V4, and the seventh fully expanded leaf at V7. The leaves were cleaned and then scanned according to the procedures described by Romualdo *et al*. (2014). The zero N level inhibited plant development; therefore, the third fully expanded leaf was used as indicative leaf.

The leaf blades were digitized at 1,200 DPI, in a high-resolution scanner (model HP G2710), identified according to nitrogen doses, and stored in tiff format (without compression), for further processing, in which, image characteristics were extracted constricting the information. A vector of characteristics represented the desired information instead of the original image. As a hypothesis for the study, characteristics related to the visible spectral indexes extracted from the images were regarded as information desired to classify the dose of N deficiency in the plant.

Equations 1 to 4 calculate these characteristics (BAESSO *et al*., 2007):

$$Eg = \frac{2Rg - Rr - Rb}{Rr + Rg + Rb} \tag{1}$$

$$Nr = \frac{Rr}{Rr + Rg + Rb} \tag{2}$$

$$Ng = \frac{Rg}{Rr + Rg + Rb} \tag{3}$$

$$Rgr = \frac{Rg}{Rr} \tag{4}$$

where,

Eg = excess of green;

Rg = rate of pixels in the green band;

Rb = rate of pixels in the blue band;

Rr = rate of pixels in the red band;

Nr = normalized red;

Ng = normalized green and

Rgr = green-red ratio.

The characteristics were calculated in blocks of the original image that represented the leaf reflectance. A computer program developed and implemented in MATLAB® R2013a cut images into blocks of 9×9, 20×20 and 40×40 pixels (BAESSO *et al*., 2007). Each dimension consisted of 10 blocks cut from the previously selected region (two at each cardinal point and two at the center of the leaf excluding the edges and the central vein). The total of 320 blocks for each studied developmental stage (4 doses of N x 2 sampled leaf blades from each dose for scanning x 4 replicates x 10 blocks) were separated into classes corresponding to doses of N.

The characteristic vector was the input variable of a classifier to discriminate the doses of N. Each color index was individually assessed, and the indexes were also combined, resulting in 15 possible index combinations as shown in Table 1.

The last step of the analysis was the development of an algorithm for the elaboration and evaluation of the statistical classifier. Gonzales and Woods (2008) described the statistical classifier evaluated for the statistical classification and evaluation of the best feature vector among the four classes corresponding to the nitrogen doses in the plant, which is composed of a set of discriminating functions (Equation 5):

$$d_j(X) = \ln P(W_j) - \frac{1}{2}\ln(\det C_j) - \frac{1}{2}\left[(X - m_j)^T C_j^{-1}(X - m_j)\right] \tag{5}$$

where,

d_j = discriminating function of class j;

C_j = covariance matrix of class j;

det C_j = determinant of the covariance matrix of class j;

X = characteristic vector;

Table 1 - Characteristic vectors (X) evaluated in the development of the classifiers to discriminate the doses of N in the leaf blade of maize plants

One index	Two indexes	Three indexes	Four indexes
X = Eg	X = Eg and Nr	X = Eg, Nr, Ng	X = Eg, Nr, Ng, Rgr
X = Nr	X = Eg and Ng	X = Eg, Nr, Rgr	
X = Ng	X = Eg and Rgr	X = Eg, Ng, Rgr	
X = Rgr	X = Nr and Ng	X = Nr, Ng, Rgr	
	X = Nr and Rgr		
	X = Rgr and Vg		

Eg = excess of green; Nr = normalized red; Ng = normalized green; Rgr = green-red ratio

m_j = rate vector of class j;

n = characteristic vector size;

$P(W_j)$ = a priori probability of class j; and

T = symbol representing the transpose of a matrix.

This work considered the occurrence of four doses of nitrogen. According to equation 6,

$$d_j(X) = -\frac{1}{2}\ln(\det Cj) - \frac{1}{2}\left[(X - m_j)^T C_j^{-1}(X - m_j)\right] \quad (6)$$

Four discriminating functions were developed, one for each class, so that an unknown vector X was assigned to class j, which presented the highest value for the discriminating function $d_j(X)$. The classification error was estimated by cross-validation leaving one out (CONGALTON; GREEN, 2009), thus an observation was separated and the discriminating functions were elaborated with the remaining data (39 samples), and then, the separate observation was classified. This procedure was repeated and evaluated successively for each sampled block size, for all of the 40 observations (4 treatments with 10 replicates). In addition, an error matrix was elaborated to estimate classification errors, as described in Congalton and Green (2009).

The generated error matrix enabled the classification of omission errors (EO) corresponding to the images not classified in the belonging class, and the inclusion errors, which included images to a not-belonging class (BAESSO *et al.*, 2007).

The classifier performance measurement was determined by the global accuracy rate in classification and by the Kappa coefficient.

Equation 7 - global accuracy rate (GAR) of the classifier:

$$GAR = \frac{CC}{n_t} x100 \quad (7)$$

where,

GAR = global accuracy rate;

CC = number of correctly classified samples; and

Nt = total number of samples.

According to Everitt and Dunn (2001), the Kappa coefficient (K) is used in this study to measure the confidence of classification. The values generated by the Kappa range up to 1.0 and the image classification was evaluated as follows: 0 - 0.20 = not trust; 0.21 - 0.40 = low; 0.41 - 0.60 = moderate; 0.61 - 0.80 = trust and 0.81 - 1.0 = worthy trust.

Equation 8 - estimate K:

$$\hat{K} = \frac{n_t \sum_{i=1}^{c} x_{ii} - \sum_{i=1}^{c} x_{i\oplus} x_{\oplus i}}{n_t^2 - \sum_{i=1}^{c} x_{i\oplus} x_{\oplus i}} \quad (8)$$

where,

$\hat{K}$ = Estimation of Kappa coefficient;

X_{ii} = Value in line i and column i (diagonal) of the error matrix;

X_{i0} = Total of line i (inclusion error);

X_{0i} = Total of column i (error of omission); and

n_t = Total number of doses.

The Z test at 5% tested the difference between both classifiers (CONGALTON; GREEN, 2009) in order to define the best spectral indexes of the classification.

Equation 9 - calculated Z value:

$$Z = \frac{\hat{K}_1 - \hat{K}_2}{\sqrt{\mathrm{var}(\hat{K}_1) + \mathrm{var}(\hat{K}_2)}} \quad (9)$$

where,

Z = Calculated Z value;

$\hat{K}_1$ = Kappa coefficient 1;

$\hat{K}_2$ = Kappa coefficient 2;

Var ($\hat{K}_1$) = Kappa coefficient variance 1; and

Var($\hat{K}_2$) = Kappa coefficient variance 2.

Equations 10, 11, 12, 13 and 14 (HUDSAM; RAMM, 1987) determined the Kappa coefficient variance.

$$\mathrm{var}(\hat{K}) = \frac{1}{n_t}\left[\frac{\theta_1(1-\theta_1)}{(1-\theta_2)^2} + \frac{2(1-\theta_1)(2\theta_1\theta_2 - \theta_3)}{(1-\theta_2)^3} + \frac{(1-\theta_1)^2(\theta_4 - 4\theta_2^2)}{(1-\theta_2)^4}\right] \quad (10)$$

where,

$$\theta_1 = \frac{1}{n_t}\sum_{i=1}^{c} x_{ii}$$

$$\theta_2 = \frac{1}{n_t^2}\sum_{i=1}^{c} x_{i\oplus} x_{\oplus i}$$

$$\theta_3 = \frac{1}{n_t^2}\sum_{i=1}^{c} x_{ii}(x_{i\oplus} + x_{\oplus i})$$

$$\theta_4 = \frac{1}{n_t^3}\sum_{i=1}^{c}\sum_{j=1}^{c} x_{ij}(x_{j\oplus} + x_{\oplus i})^2$$

The foliar nitrogen analysis according to Bataglia *et al.* (1983) was performed at V4 and V7 for the validation of the system. After each collection, the plants were washed, conditioned in paper bags and placed in an oven at 65 ºC for 72 hours to determine dry mass (shoot and root). The relative production at 90% was calculated based on the total dry matter production, as well as the determination of the critical level of N in the nutrient solution and leaf. Regression studies were carried out for significance of the results, and the correlation of the nitrogen content with the dry mass production was established using SAS 9.2 software (SAS INSTITUTE, 2012).

RESULTS AND DISCUSSION

Nitrogen contents (N) in the plant tissue of indicative leaf blades at V4 and V7 increased significantly ($p<0.01$) with incremental doses of N in the nutrient solution (Figure 1a). Dry mass production had a positive and significant correlation with the contents (Figure 1b). The inadequate nitrogen supply significantly compromised maize development (Figures 1 and 2), hence the plant biomass production value. The N characteristic deficiencies in the plant were observed in the blades of the old leaves with chlorosis that evolved to dry, and necrosis of the vegetal tissue progressing along the main vein. Ferreira (2012) and Gondim *et al.* (2010) found similar results.

The N sufficiency range was established at the developmental stages based on the equations described in Figure 2, ranging from 7.6 to 15.0 mmol L^{-1} at V4 and from 9.4 mmol L^{-1} to 16.7 mmol L^{-1} at V7. Over the maximum stablished values (Figure 2), the probability of response to the addition of nitrogen in the solution is very low in sufficient ranges. The minimum concentration required to reach 90% of maximum yield was higher at V7 (9.4 mmol L^{-1}). Therefore, small decreases in N concentration may compromise the dry mass production of the plant. The leaf contents of N, to reach 90% of the dry mass for the internal critical level at V4, were 37.2 g kg^{-1} and 50.6 g kg^{-1}. Plants with fast early growth, such as maize, require nitrogen in a short period, and if the requirements are not supplied, there is damage in the initial growth, with effects on grain production (ALMEIDA *et al.*, 2013).

The obtained results allow us to infer there was a significant variation among the foliar contents of the treatments according to the applied N rates and to recognize a lack of nitrogen by image analysis based on spectral indexes.

Tables 2 and 3 present the global accuracy rate (GAR) and Kappa coefficient of developed classifiers for indicative images of maize leaf, at the studied developmental stages, considering the three dimensions of image blocks (9 × 9, 20 × 20 and 40 × 40 pixels).

The results from the combined spectral indexes showed significant differences compared to individual analysis. When combined, they presented a better determination of N doses, with a global accuracy probability over 65% (Table 1) and 80% (Table 2) at the V4 and V7 stages, respectively.

Independent of block size, the combinations of normalized red (Nr), normalized green (Ng) and green and red ratio (Rgr) among the indexes presented higher GAR (80, 78 and 68%, respectively) and the Kappa indexes were trust and moderate (0.73, 0.70 and 0.57, respectively). Whereas for the V7 stage (Table 3), despite not reaching the highest GAR (93, 85 and 83%, respectively), the Kappa indexes worthy trust and trust

Figure 1 - N content in the indicative leaf (a) and correlation of N contents with dry mass (b), as a function of N concentration in the nutrient solution, in two developmental stages (V4 and V7 phenological stages). ** Significant at 1%

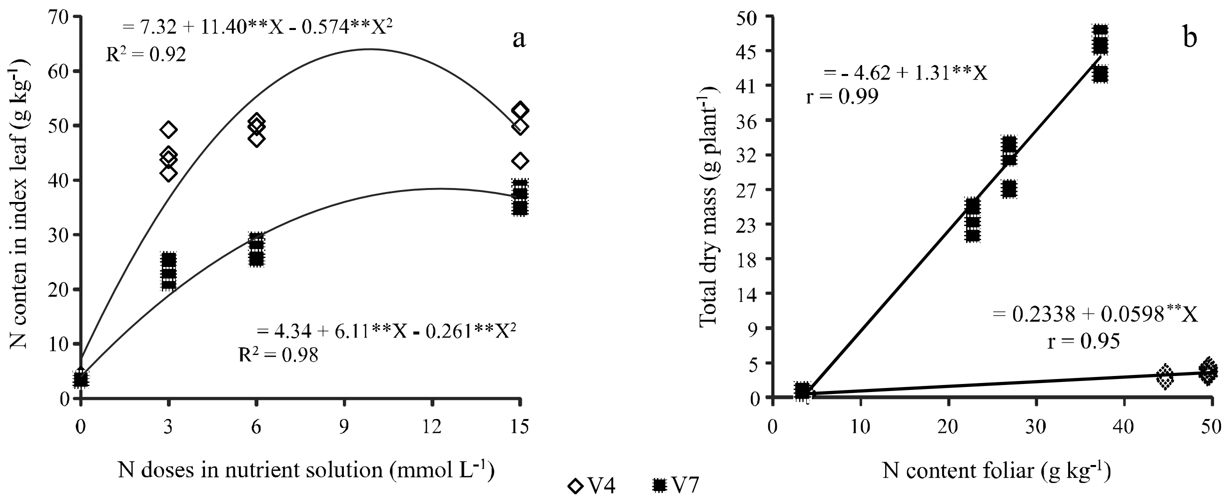
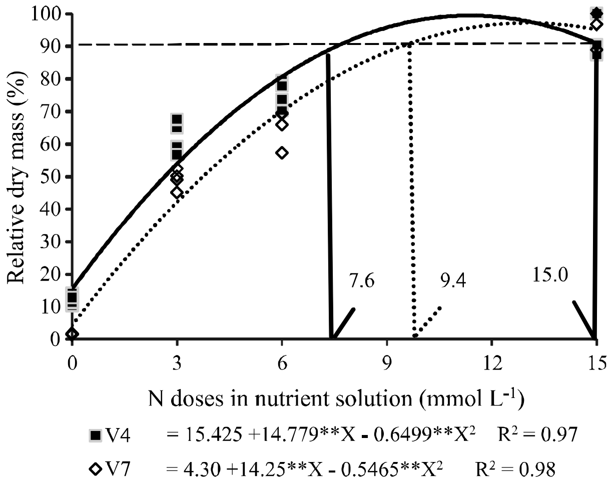


Figure 2 - Relative dry mass and critical level of N as a function of N concentration in the nutrient solution (a) and as a function of the N content in the indicative leaf (b), at different times of development. **Significant at 1%



(0.90, 0.80, 0.77, respectively), showing no significant difference in relation to the indexes with higher GARs. Abrahão *et al.* (2013) and Baesso *et al.* (2007) observed that the combinations among the spectral indexes promoted a better determination of N foliar contents and chlorophyll in the common bean, by bands and spectral vegetation indexes. Romualdo *et al.* (2014) studied through image analysis the different extraction methods for the recognition of nitrogen deficiency in leaves of maize at the V4 phenological stage, and verified that the classification increased in quality when spectral patterns were added.

Sena Júnior *et al.* (2008) worked with topdressing fertilization levels for wheat (0, 30 e 60 kg ha^{-1}), and used a spectral index (red, green, infrared bands and combination of four spectral bands indexes) extracted from canopy digital images evaluated at 8, 14 and 30 days of nitrogen fertilization. The authors concluded that the indexes discriminate the N doses in the wheat canopy after 8 days of N topdressing. Silva *et al.* (2014) studied different extraction methods for detecting magnesium deficiency in corn leaves at the V4 stage, and verified the color information associated with the texture characteristic showed a higher percentage of global accuracy (98%) and Kappa (0.9) compared to gray scale images (GAR 50% and Kappa 0.7). However, Silva Junior *et al.* (2008) verified the green band was more efficient in relation to the foliar nutritional estimate, the foliar N content and the DM productivity than the red band.

Table 2 - Global accuracy rate (GAR) and Kappa index for each spectral index and combinations, for the different image block dimensions of the indicative leaf at the V4 stage

Spectral Indexes	9×9 pixels		Spectal Indexes	20×20 pixels		Spectal Indexes	40×40 pixels	
	GAR%	Kappa		GAR%	Kappa		GAR%	Kappa
Nr, Ng, Rgr	80	0.73 a	Nr, Ng, Rgr	78	0.70 a	Nr, Ng, Rgr	68	0.57 a
Eg, Nr, Ng, Rgr	75	0.67 ab	Eg, Ng, Rgr	78	0.70 a	Ng	60	0.47 a
Eg, Nr, Ng	73	0.63 ab	Eg, Nr, Ng	78	0.70 a	Eg and Rgr	60	0.47 a
Eg, Ng, Rgr	70	0.60 ab	Ng and Rgr	78	0.70 a	Ng and Rgr	60	0.47 a
Eg and Ng	65	0.53 abc	Nr and Rgr	78	0.70 a	Eg	58	0.43 a
Eg and Rgr	63	0.50 bc	Eg and Rgr	78	0.70 a	Rgr	58	0.43 a
Nr and Ng	63	0.50 bc	Nr and Ng	75	0.67 ab	Eg e Nr	58	0.43 a
Nr and Rgr	63	0.50 bc	Eg and Nr	75	0.67 ab	Nr e Ng	58	0.43 a
Ng and Rgr	63	0.50 bc	Eg and Ng	68	0.57 ab	Nr e Rgr	58	0.43 a
Rgr	60	0.47 bc	Eg, Nr, Ng, Rgr	65	0.53 ab	Eg, Nr, Ng	58	0.43 a
Eg and Nr	60	0.47 bc	Eg	65	0.53 ab	Eg, Ng, Rgr	58	0.43 a
Eg	48	0.30 c	Ng	65	0.53 ab	Eg, Nr, Ng, Rgr	58	0.43 a
Ng	48	0.30 c	Nr	55	0.40 b	Eg e Ng	55	0.43 a
Nr	45	0.27 c	Rgr	50	0.33 b	Nr	40	0.20 b

Kappa coefficients followed by the same letter do not differ in the line through Z test, at 5% probability and followed by distinct letters differ significantly through Z test, at 5% probability. Eg = excess of green; Nr = normalized red; Ng = normalized green; Rgr = green-red ratio

Table 3 - Global accuracy rate (GAR) and Kappa index for each spectral index and combinations, for the different image block dimensions of the indicative maize leaf at the V7 stage

Spectral Indexes	9×9 pixels		Spectral Indexes	20×20 pixels		Spectral Indexes	40×40 pixels	
	PAG%	Kappa		PAG%	Kappa		PAG%	Kappa
Eg, Ng, Rgr	95	0.93 a	Nr	90	0.87 a	Eg e Nr	90	0.87 a
Ng, Rgr	95	0.93 a	Eg and Nr	88	0.83 ab	Eg e Rgr	90	0.87 a
Eg and Rgr	95	0.93 a	Eg, Nr, Ng	88	0.83 ab	Ng e Rgr	90	0.87 a
Eg, Nr, Ng	93	0.90 a	Eg, Vern, Ng, Rgr	88	0.83 ab	Nr e Ng	90	0.80 a
Nr, Ng, Rgr	93	0.90 a	Nr and Ng	88	0.73 ab	Nr	88	0.83 a
Eg and Nr	90	0.87 a	Nr, Ng, Rgr	85	0.80 ab	Nr and Rgr	85	0.80 a
Eg, Nr, Ng, Rgr	90	0.87 a	Eg and Rgr	83	0.77 ab	Eg, Nr, Ng	85	0.80 a
Nr and Ng	90	0.80 ab	Ng and Rgr	83	0.77 ab	Eg, Ng, Rgr	85	0.80 a
Nr	85	0.80 ab	Eg, Ng, Rgr	83	0.77 ab	Nr, Ng, Rgr	83	0.77 a
Nr and Rgr	85	0.80 ab	Nr and Rgr	80	0.73 ab	Eg, Vern, Eg, Rgr	83	0.77 a
Eg	73	0.63 b	Eg	75	0.67 b	Eg, Ng	63	0.50 b
Ng	73	0.63 b	Ng	75	0.67 b	Eg	60	0.47 b
Eg e Ng	73	0.63 b	Vern	55	0.40 b	Ng	60	0.47b
Rgr	48	0.30 c	Rgr	50	0.33 b	Rgr	58	0.43b

Kappa coefficients followed by the same letter do not differ in the line through Z test, at 5% probability and followed by distinct letters differ significantly through Z test, at 5% probability. Eg = excess of green; Nr = normalized red; Ng = normalized green; Rgr = green-red ratio

Gonzalez and Woods (2008) reported that three visible color channels provide relevant image information. However, the isolate analysis may result in a loss of important information among these colors in the same pixel for the 3 channels. In this sense, the colorful image represents an important attribute to differentiate changes that occur in the leaf surface as a function of nutrient deficiency, even when these deficiencies are latent.

In this study, the statistical classifiers that obtained a Kappa value greater than 0.80 were considered worthy trust. Table 4 shows the occurrence frequency of Kappa classifiers equal to or greater than 0.80 for each image acquisition period and each combination of spectral indexes.

According to Table 4, there was no occurrence of excellent Kappa at V4; however, among the 15 spectral indexes studied, 10 combinations among them presented better performance in the classification, confirming that when the indexes are combined it is possible to distinguish nitrogen nutritional standards in the plant. The V7 stage best presents these distinctions.

The obtained results indicate a lower nitrogen at V4, as observed in the critical concentrations of N at this stage (Figure 2), but still do not show differences as development progresses. However, as the demand for N

Table 4 - Frequency of occurrence for Kappa classifiers equivalent to or higher than 0.80 at the developmental stages V4 and V7 for each combination of spectral indexes used as characteristic vector

Spectral indexes	V4	V7*
Eg	0	0
Nr	0	3
Ng	0	0
Rgr	0	0
Eg and Nr	0	3
Eg and Ng	0	0
Eg and Rgr	0	2
Nr and Ng	0	3
Nr and Rgr	0	2
Ng and Rgr	0	2
Eg, Nr, Ng	0	3
Eg, Nr, Rgr	0	3
Eg, Ng, Rgr	0	2
Nr, Ng, Rgr	0	2
Eg, Nr, Ng, Rgr	0	2

Eg-excess of green; Nr-normalized red; Ng- normalized green; Rgr-green-red ratio. *Frequency of maximum occurrence equivalent to 3 (referring to the three block dimensions) increased, a small reduction of N levels in the nutrient solution under 9.4 mmol L^{-1} at V7 (Figure 2), allowed for a better class discrimination by image analysis.

The image block dimension did not influence the classification of nitrogen patterns when compared within the same time period, as the best results did not differ through Z test at 5% probability (Table 5). However, when compared between seasons, the 9 × 9 and 40 × 40 blocks at V7 were significantly higher. Searching for a smaller and standard block size, which implies a shorter image processing time, the 9 × 9 pixel block provides sufficient information for an adequate classification.

Table 5 - Kappa coefficient of the best classification results, at the developmental stages V4 and V7, for the blocks of 9 × 9; 20 × 20 and 40 × 40 "pixels"

Block dimension	Developmental stages	
	V4	V7
9 × 9 "pixels"	0.73 Ab	0.93 Aa
20 × 20 "pixels"	0.70 Aa	0.87 Aa
40 × 40 "pixels"	0.57 Ab	0.87 Aa

Kappa coefficients followed by the same lowercase letter do not differ in the line through Z test, at 5% probability; Kappa coefficients followed by the same capital letter do not differ, in the column, through Z test at 5% probability

CONCLUSIONS

1. Image analysis using visible spectral indexes allowed for the identification of nitrogen deficiency in maize plants at the V4 and V7 developmental stages;
2. The use of combined spectral indexes in the classifier characteristic vector provided better classification performance;
3. The highest differentiations among classes occurred at stage V7;
4. The 9 × 9 pixel image block was the most adequate to distinguish classes corresponding to the nitrogen rates. For the conditions in this study, the combination of Nr, Ng and Rgr indexes allowed a higher GAR and Kappa index, which was very trust and worthy trust, obtaining 80% and 0.73 at V4 and 93% and 0.90 at V7, respectively;
5. The increase in N doses in the nutrient solution promoted higher N content in the indicative leaves at both developmental stages;
6. N supply for maize implies linear responses in dry mass production, based on the concentrations used in the nutrient solution, at the V4 and V7 developmental stages, according to the conditions in this study;
7. At V4 and V7, the external critical levels of N were 7.6 and 9.4 mmol L^{-1}, respectively.

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