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RESEARCH

A Diagnostic Tool for Magnesium Nutrition in Maize Based on Image Analysis of Different Leaf Sections

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ABSTRACT

The nutritional status of maize (*Zea mays* L.) can be diagnosed by chemical analysis of leaves, which is very slow, or by visual diagnosis of deficiency symptoms, which is dependent on observer experience. The artificial visual system (AVS) is a technology to identify nutritional deficiencies in maize, allowing correction for nutrient supply at earlier development stages in maize. Our objective was to propose methods of artificial vision and pattern recognition to identify the concentration of magnesium (Mg) in maize plants grown in the greenhouse. Magnesium concentrations were 0.0, 0.65, 1.3, and 2.0 mM Mg, with four replications. Leaf scans were collected at V4 (four leaves fully developed), V6 (six leaves fully developed), and V8 (eight leaves fully developed) stages, and these leaves were samples for chemical assays. Such images were processed using AVS methods. Volumetric fractal dimension (VFD), Gabor wavelet (GW), and VFD with canonical analysis (VFDCA) were techniques used by the AVS to extract deficiency characteristics in the leaf images. The increase of Mg in the nutrient solution caused an increase in the Mg concentration in leaves, resulting in typical visual symptoms. The AVS method was able to identify all levels of deficiency, scoring 75.5% of rights in images of the middle section of leaves in the VFDCA method, in color scans of V4 leaves. The AVS was efficient at diagnosing Mg concentrations in leaves of maize during the V4 stage.

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Abbreviations: AVS, artificial visual system; GW, Gabor wavelet; IL, index leaf; OL, old leaf; VFD, volumetric fractal dimension; VFDCA, volumetric fractal dimension with canonical analysis.

Because plant nutrients have specific and essential roles in metabolism (Malavolta, 2006), the lack of any nutrient causes disturbance in plant metabolism (Epstein and Bloom, 2004). Recognizing symptoms of nutritional deficiency allows for correcting them more efficiently and reducing the negative effects to the environment (Sarcinelli et al., 2004). Methods to measure the concentration of chemical elements in plant leaves are expensive and time consuming (Guimarães et al., 1999). In addition, if the recognition occurs during the later stages of plant development, such information is not useful to correct the deficiency that production cycle (Wu et al., 2007). A rapid and inexpensive way to identify such deficiency is through visual diagnosis, but its precision is limited to the experience of the observer (Baesso et al., 2007). Since both chemical analysis and visual diagnosis have disadvantages, this study presents an approach to assess the plant nutritional status for Mg through analysis of leaf images. In this approach, artificial vision methods are used to extract features

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from leaf images and pattern recognition methods are then used to classify the leaf images for nutrient disorder.

The AVS is a computer system in which a set of methods and techniques are able to interpret or assess images automatically or semiautomatically (Punam and Udupa, 2001), and the artificial vision is an open area and developing (Niblack, 1985). The use of digital images in precision agriculture is not recent and many studies have shown promising results. Vooren and Heijden (1993) used digital image analysis techniques to determine the dimensions of plant organs. Karcher and Richardson (2003) determined lawn color using digital image analysis. Baesso et al. (2007) used digital image analysis to detect nitrogen levels in bean (*Phaseolus vulgaris* L.) plants. Sena et al. (2008) evaluated the discrimination of three nitrogen levels on wheat (*Triticum aestivum* L.) plants using digital images and a portable chlorophyll meter (SPAD), and found that using the classification of artificial vision techniques were better than the SPAD. Abraha et al. (2009) developed multivariate classifiers using spectral indices for the determination of four nitrogen levels in Tanzania grass (*Panicum maximum* Jacq.). Backes et al. (2010) used the digital image analysis compared with the chlorophyll meter to assess the nutritional status of nitrogen in zoysiagrass (*Zoysia japonica* Steud.) in area fertilized with five levels of sewage sludge.

Magnesium deficiency causes typical symptoms such as light green veins in older, more mature leaves, followed by yellow and possibly brown areas with necrosis (Malavolta, 2006). The AVS has potential to identify these characteristics and relate them to one or more nutrient deficiencies in the plant.

The objective of this work was to propose methods of artificial vision and pattern recognition, based on image analysis of different leaf sections, that would best identify the concentration of Mg in maize (*Zea mays* L.) plants grown hydroponically in the greenhouse.

MATERIALS AND METHODS

Greenhouse Experiment

The experiment was done in the Animal Science and Food Engineering College of University of São Paulo at the Pirassununga-São Paulo. The crop tested was maize, hybrid DKB 499, grown in a hydroponic system in a greenhouse. Seeding was done in plastic trays filled with washed sand. After emergence, two plants were transferred to each pot (3.6 L) and supported by a foam on top of the pot filled with Hoagland and Arnon (1950) nutrient solution (50% full volume) modified to achieve the target Mg concentration, during 5 d. After this time span, pots were completely filled with the nutrient solution, and completely replaced weekly. The pH was monitored between 5.0 and 6.0 pH units, and temperature averaged approximately 28°C. Each pot had air bubbling during 10 s at each 30-s interval.

Four concentrations of Mg were tested: 0.0 mM, 0.65 mM (33% of full dose), 1.3 mM (66% of full dose), and 2.0 mM (of full dose—100%) Mg. These concentrations were chosen arbitrarily to provide a range of leaf Mg to work with.

Plant and leaf images were sampled at three stages of maize development: V4 (plants with four leaves fully developed), V6 (plants with six leaves fully developed), and V8 (plants with eight leaves fully developed). According to Fancelli (1986), at V4 plant occurs the definition of the productive potential, at V6 the definition of the number of seeds in the ear, and at V8 the definition of the number and size of the ear.

Experimental design was fully random in a 4-by-3 factorial (four Mg concentrations and three sampling events) with four replications. In each collecting period established, 16 pots were sampled (samples destructive). Sampled material was split into (i) index leaf (IL) of the growing stage (V4 = leaf 4, V6 = leaf 7, and V8), and (ii) old leaf (OL), both to image capture and chemical analysis.

For chemical analysis, all material was washed, dried in an oven with air circulation at 65°C, ground, and saved in plastic bags for further nutrient analyses, according to methodology described in Bataglia et al. (1983). Samples were solubilized with nitric–perchloric acid for determination of Mg in OL and IL.

Statistical Analysis

Statistical data analysis was accomplished using variance analysis and Tukey test at 5% probability. Such analysis was applied to data from Mg concentration in plants (g kg^{-1}), dry mass in shoots, and dry mass in roots. The mathematical model used was:

$$Y_{ijk} = m + E_i + N_j + \mathrm{EN}_{ij} + e_{ijk}$$

where Y_{ijk} is the value measured in the unit subjected to treatment ij at replication k, m is the overall average of the experiment; E_i is the effect of maize stage of development; N_j is the effect of Mg concentrations applied; and e_{ijk} is the effect of uncontrolled factors at the unit subjected to treatments ij at replication k. In cases where the F test was significant ($P \leq 0{,}05$) only to N_j, only one polynomial regression was performed on all plant stages of development. In cases where the F test was significant ($P \leq 0{,}05$) for NE_{ij}, that is, in which there was interaction between the Mg concentrations and stages of plant development, the unfolding had the objective to study the concentrations inside E_i. In such cases, one regression analysis was performed to each development stage (three in total).

Artificial Visual System

The images were processed by artificial vision, using the traditional four-step approach: acquisition, image segmentation, feature extraction, and classification/identification (Bruno, 2000; Gonzalez and Woods, 1993).

The acquisition step is responsible for the image digitalization. Since the digitalization in the presented system is made by the operator using a conventional scanner, this step is not integrated into the proposed vision system. During the image segmentation step, the digitalized image is processed to be analyzed by the system. The image processing step consists of two parts. In the first part, the leaves' images are split and segmented from the background, after which they are oriented (down to top). In the second part, windows (areas of interest on the image) are extracted from the leaves, which are used in the next step. The feature extraction step is responsible for the analysis

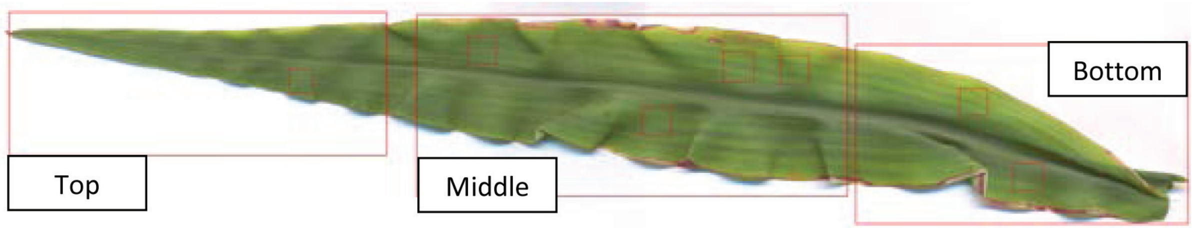

Figure 1. Separation of leaves for image analysis into three parts: bottom, middle, and top.

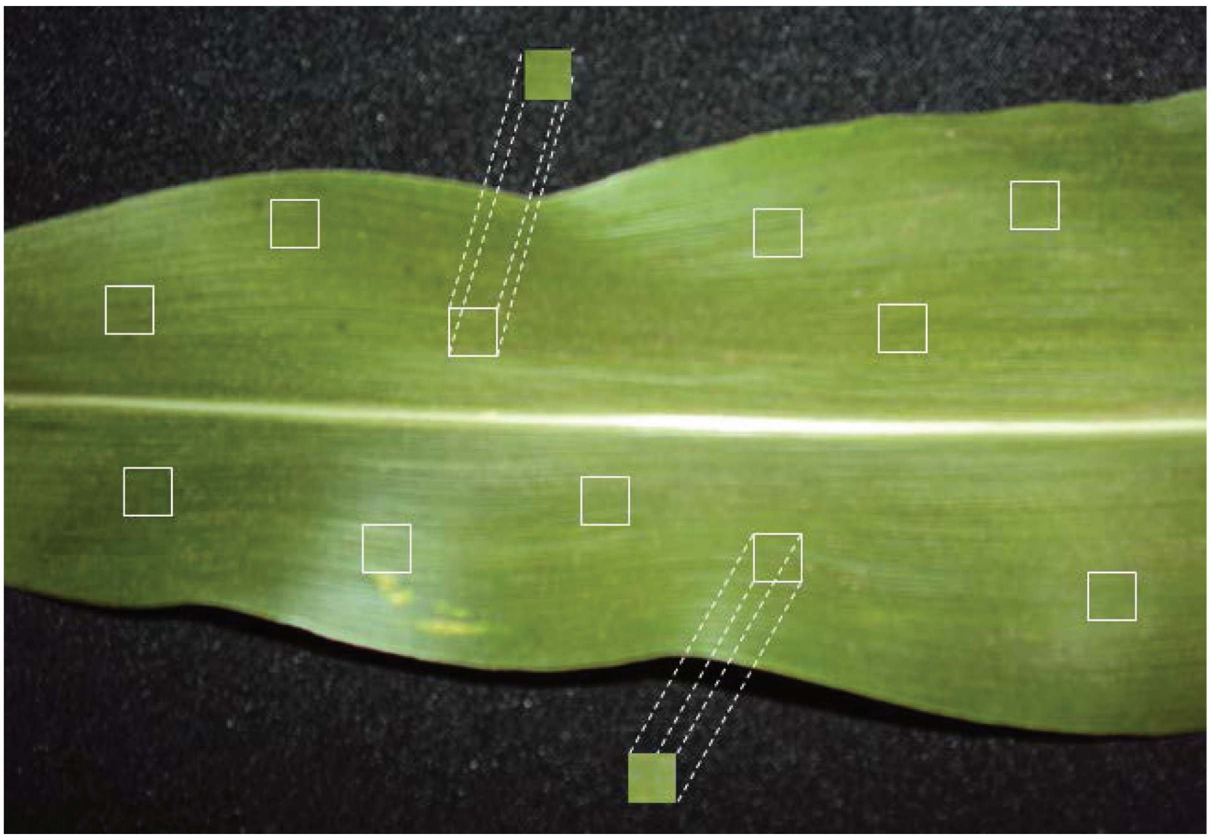

Figure 2. Segmentation process (Backes et al., 2009).

of the windows. For each window, a signature is extracted to characterize the leaf. Finally, the last part is the classification/identification step, where the pattern recognition algorithms perform the classification of the leaves based on the feature vector extracted in the previous step.

The IL and OL leaves were cut at the base and then were scanned at 1200-dpi resolution using a conventional flatbed scanner (HP Scanjet 3800). Subsequently, the images were stored as TIFF extension for further processing and extraction of windows by the methods of artificial vision presented below and subsequently classified.

At each timing established for the study, one IL and OL were removed from each plant. Each experimental unit grew two plants, so they were removed by Treatment 8 IL and 8 OL in each collection for scanning.

Three sectors of each scanned leaf were used: bottom, middle, and top (Fig. 1), for IL and OL, summing up 50 color images and 50 grayscale images for each. In each part of the leaf were extracted windows of 80 by 80 pixels of the leaf surface. Each window was oriented horizontally and stored in uncompressed format. The leaf texture window technique was proposed in Casanova et al. (2009) and Rossatto et al. (2011). The main idea of this approach is analyzing the leaf micro-texture so that the 80-by-80-pixel windows can isolate the micro-texture features, allowing that the macro-texture does not interfere into the texture analysis. In addition, as shown by Casanova et al. (2009) and Rossatto et al. (2011), the window approach allows sample extraction for different positions of the leaf and allows discarding windows that are completely different within homogeneous regions, that could contain outliers, such as leaf defects, insect-damaged areas, etc.

The segmentation process was used to discard the image background and artifacts (damage, holes, etc.) and also to isolate target areas that showed nutrient deficiency symptoms (Fig. 2). Image analysis was focused mainly on color and texture, which were the visual characteristics best related to nutrient content (Rossatto et al., 2011).

Different methods of feature extraction were evaluated: volumetric fractal dimension (VFD), Gabor wavelet (GW), and VFD with canonical analysis (VFDCA), to select a best method for the

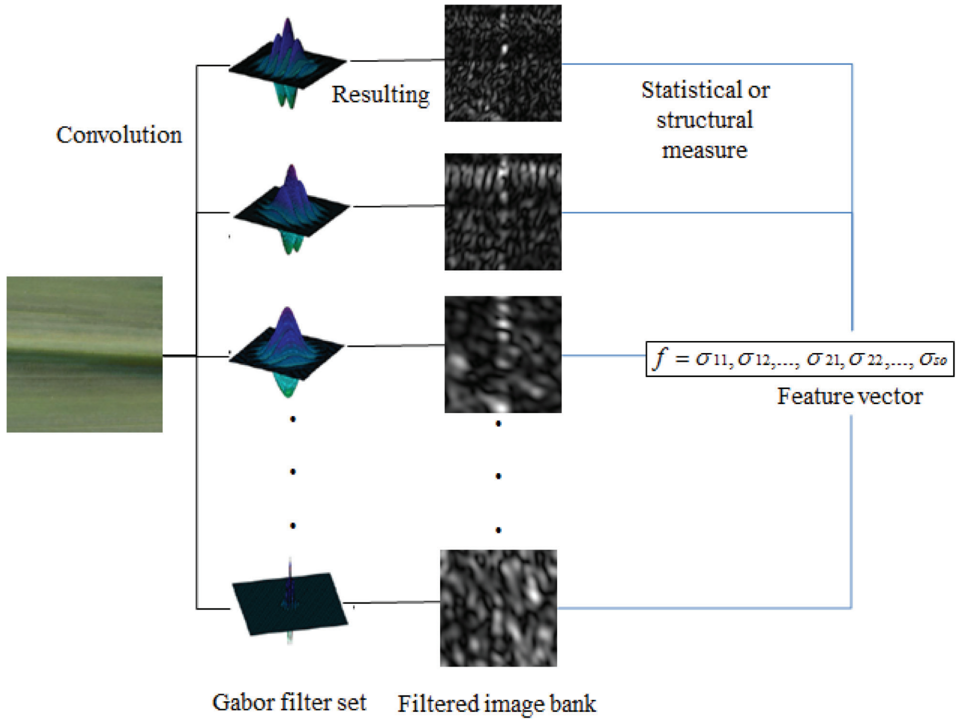

Figure 3. Process of feature extraction (adapted from Zuñiga and Bruno, 2010).

unambiguous identification of the nutrient deficiency classes, concentrations of Mg in the three leaf segments. These methods were chosen based on the good results obtained in the leaf texture analysis. In Bruno et al. (2008), Casanova et al. (2009), Backes et al. (2009), Rossatto et al. (2011), and Backes and Bruno (2013), the authors compared state-of-the-art texture methods for leaf identification and the best results were achieved by them.

The VFD method worked on binary images as proposed by Backes et al. (2009), in which the image signature is calculated for all values of reE, where E is the set of Euclidean distances for a maximum radius $r_{\max}$. In this technique the radius ranges from 1 to 20:

$$E = 1, \sqrt{2}, \sqrt{3}, \ldots, r_{\max}$$

$$(r_{\max}) = \begin{matrix} \log V(1), \log V\left(\sqrt{2}\right), \\ \log V\left(\sqrt{3}\right), \ldots, \log V\left(r_{\max}\right) \end{matrix}$$

The transformed Gabor bidimensional is a Gaussian function modulated in a sine wave oriented with a frequency W and a direction θ, and its bidimensional form in the space $g(x,y)$ and frequency $G(u,v)$ is given by the following equations:

$$g(x,y) = \left(\frac{1}{2\pi\sigma_x\sigma_y}\right)\exp\left[-\frac{1}{2}\left(\frac{x^2}{\sigma_x^2}+\frac{y^2}{\sigma_y^2}\right)\right] + 2\pi jWx$$

$$G(u,v) = \exp\left\{-\frac{1}{2}\left[\frac{(u-W)^2}{\sigma_u^2}+\frac{v^2}{\sigma_v^2}\right]\right.$$

The transformed Gabor can be adapted as a wavelet and in this case the equations are used as a mother wavelet. Next, a filter dictionary can be obtained by dilatations and rotations of $gz(x,y)$ through the function proposed by Manjunath and Ma (1996):

$$g_{mn}(x,y) = a^{-m} g\left(x', y'\right)$$

where $a > 1$ and m,n are the scale and orientation, respectively, with $m = 0, 1, \ldots, M - 1$, and $n = 0, 1, \ldots, N - 1$; M is the total number of scales and N is the total number of orientations.

Figure 3 shows the process of implementing the use of extractants (adapted from Zuñiga and Bruno, 2010).

In all methods of extracting the AVS used the naive Bayes classification and the cross-validation learning method were used. Each image processing, 80% of the images were used for training and 20% for testing "blind." The classification experiment was performed considering the four concentrations of Mg deficiency. These concentrations were controlled and also validated with the chemistry analysis. The goal of the classification experiment is verifying the image analysis accuracy to detect the nutrient deficiency, classifying the groups according to both chemistry analysis and controlled concentrations of Mg.

A confusion matrix was produced to measure the number of 1 (one) windows correctly identified. And it is important to know if the classes were difficult to classify. In addition, the following indices were generated: percentage of images correctly classified or Global Percentage of Rights, which is the most important information. And Kappa index (K, trust index) was fit into the following classes: 0–0.2: not trust; 0.21–0.4: low; 0.41–0.6: moderate; 0.61–0.8: trust; 0.81–1.0: trustworthy. The Kappa index is a statistical measure of agreement or accuracy well known in Pattern Recognition (Congalton, 1991). The Kappa index is used in this work to measure the confidence of the classification.

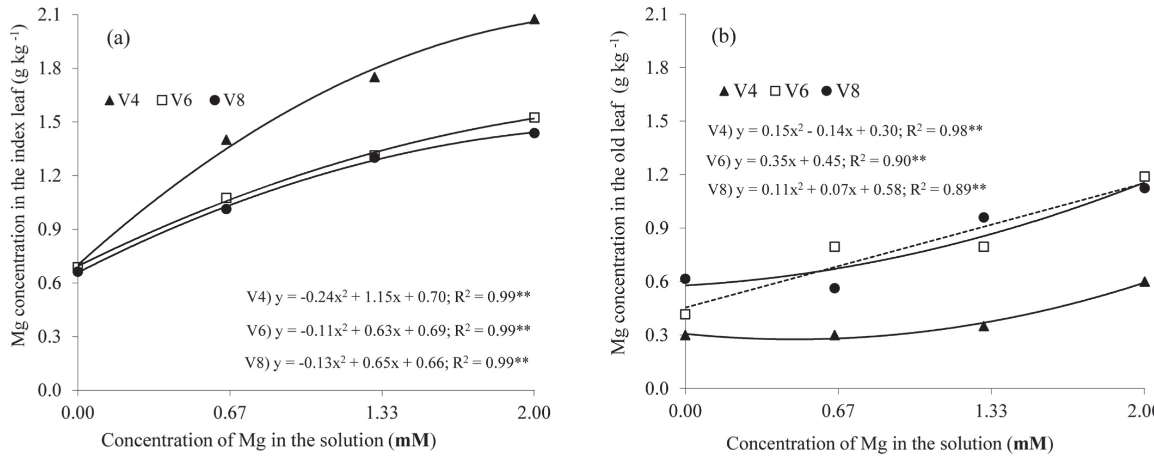

Figure 4. Magnesium concentration in the (a) index leaf and (b) old leaf of maize at the V4, V6, and V8 stages as a function of Mg concentration in nutrient solution. **Significant at 0.01 probability level.

RESULTS AND DISCUSSION

Concentration of Magnesium in Leaves

The Mg concentration both in IL and OL increased significantly ($p < 0.05$) in all stages with the increase of Mg in the solution. This relationship is expressed by the polynomial equation (Fig. 4). As Mg concentration increased, the concentration in leaves reached up to 2.08 g kg^{-1} in IL (Fig. 4a) and 0.60 g kg^{-1} in OL (Fig. 4b) at the V4 stage. As expected, leaf Mg concentration was lowest for plants at all stages of growth when grown in the 0 mM Mg nutrient solution.

The concentration of Mg in IL was greater when compared to the OL, highlighting the Mg mobility within the plant, which is rapidly translocated from mature towards the new tissues. As a consequence, the deficiency symptoms appear first in the old leaves (Epstein and Bloom, 2004). Malavolta (2006) suggests that concentration between 2.5 to 4.0 g kg^{-1} Mg is sufficient for leaf blades in corn that are located below and opposite to the corncobs and at the R1 stage. In the present study, the leaves evaluated were at stages V4, V6, and V8 (previous to the R1 stage). Therefore, the concentrations suggested by Malavolta (2006) can be used as a reference to evaluate the present results.

Visual Symptoms, Shoot Dry Mass, and Roots Dry Mass

The increase in Mg concentration in the solution resulted in an increase in its concentration in leaves. Visual symptoms in leaves, characteristic of situations with absent or undersupply of Mg, was easily observed. Plants with Mg deficiency had leaf blades with chlorosis in the interveinal area, green veins, leaf margins, and tops deformed toward the underside. These symptoms appeared first in the old leaves (Fig. 5a and 5b). The chlorosis evolved towards the tip of the leaf blade, and turned to tan and necrosed in plants with lack of Mg and those with the smallest weekly treatment solution concentration of 0.64 mM (Fig. 5c and 5d). Similar symptoms were also observed by other authors (Malavolta et al., 1997; Monteiro et al., 1995). According to Sarcinelli et al. (2004), interveinal chlorosis in plants with complete lack of Mg supply is a typical symptom of that deficiency.

The Mg deficiency symptoms were markedly related to the diminishing plant growth. The lack of Mg in nutrition solution halted the dry mass production in shoots ($p < 0.01$) and roots ($p < 0.01$) at the three stages (Fig. 6). A quadratic increase ($p < 0.01$) was observed in dry mass in shoots and roots along the increase in Mg concentration in solution (Fig. 6) in all stages. The highest dry mass production in shoots estimated by the regression equation was 5.4 (V4), 31.2 (V6), and 57.8 g kg^{-1} (V8), for concentrations 1.7, 1.5, and 1.4 mM. For roots, the highest production of dry mass was 1.5 (V4), 9.4 (V6), and 17.5 g kg^{-1} (V8), for concentrations 1.8 (V4–V6) and 1.4 mM (V8). Magnesium was essential to the photosynthesis process and to the cell activity, and therefore strongly associated with plant growth rate. As a consequence, Mg availability is indispensable to good crop performance of maize (Fancelli, 2010).

Artificial Vision System

Considering the images of all treatments in grayscale (without color information) to maize plants with levels of Mg deficiency in the greenhouse, the best results were found in 50% of middle section and 63% at the bottom of IL using the GW technique at the V4 and V8 stages. The VFDCA technique reached 57% of rights in the IL at the V6 stage (Table 1). Such results suggest that the amount of information in grayscale images is not enough to distinguish the levels of deficiency in maize leaves, grown in the greenhouse, because the number of right diagnoses was too small.

To verify the importance of the information in color in the image processing and classification, the analyses were run again using the color images (Table 2). The percentage of rights in color images was greater than in grayscale images in stages V6 and V8 but mostly in stage V4.

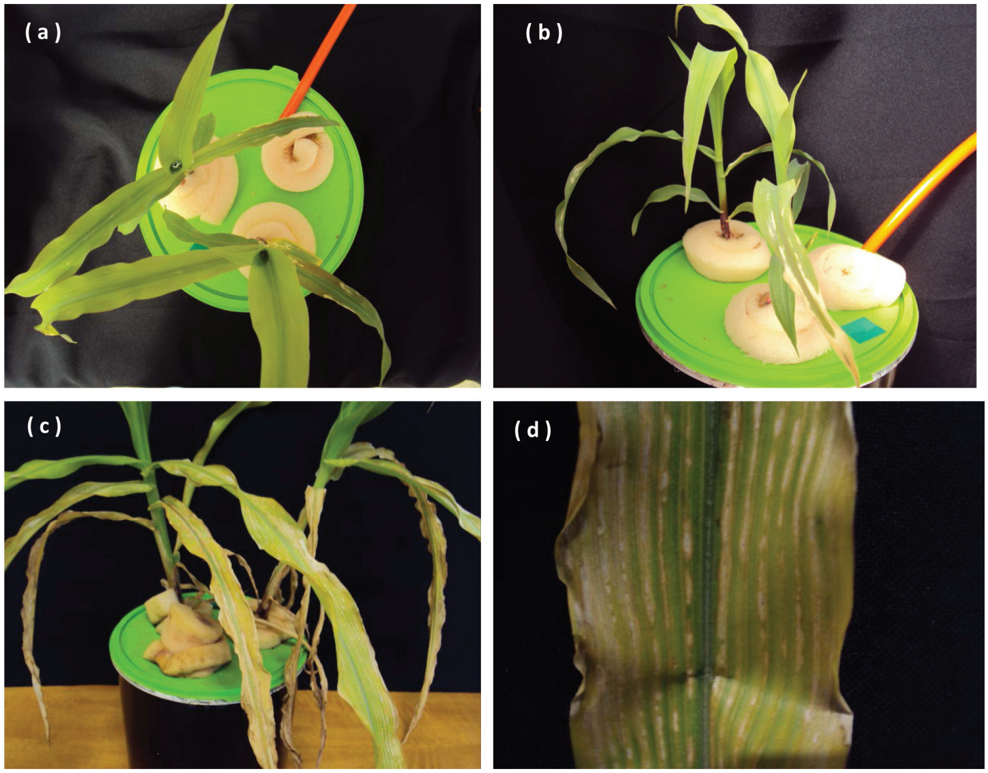

Figure 5. Symptoms of Mg deficiency in maize plants (a, b) at 22 d after plant emergence and (c, d) at the V6 stage, growing without Mg supply in the solution.

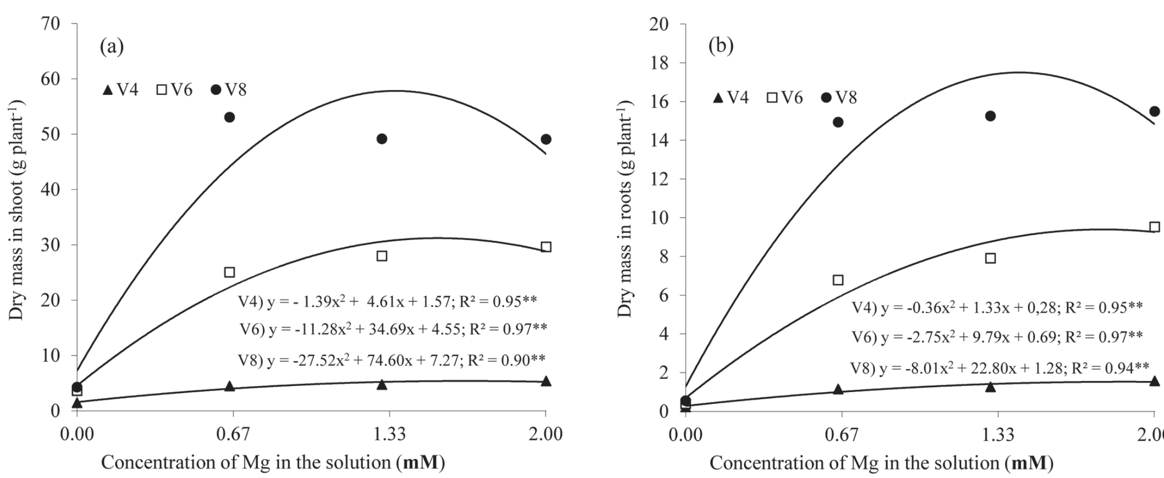

Figure 6. Dry mass in (a) shoot and (b) roots of maize at the V4, V6, and V8 stages as a function of Mg concentration in nutrient solution. **Significant at 0.01 probability level.

The best result was 75.5% of rights at the V4 stage with the VFDCA technique run in color images, showing good potential for use in identification of Mg deficiency in greenhouse-grown maize plants, through images in V4 stage.

Therefore, the middle region of the IL of maize plants in greenhouse is the best section to observe in order to identify Mg deficiency in stage V4 (75.5%), with the fraction of rights sufficient to be considered trustworthy through the Kappa index (Table 2).

In Table 3, the confusion matrix is shown for the middle section of the IL, classified using the VFDCA technique run on color images taken at stage V4. From the 50 images used, all with 0.0 mM Mg, were correctly classified by this technique. Among the images from plants with 0.65 mM Mg, 82% of images were correctly identified, 2% as 0.0 mM Mg, 10% of images as 1.3 mM Mg, and 6% as 2.0 mM Mg. These results suggest maize leaves at stage V4 with Mg deficiency were those most easily classified by the AVS, and that the leaf images from the plants grown with 1.3 mM were those more difficult to be classified. In the confusion matrix presented (Table 3), the largest number of errors occurred for the classification of the class of plants that received 1.3 mM. This is because the image windows analyzed by the AVS for the 1.3 mM

Table 1. Global Percentage of Rights (GPR) of grayscale images using the volumetric fractal dimension (VFD), volumetric fractal dimension with canonical analysis (VFDCA), and Gabor wavelets (GW) to assess leaf Mg, and corresponding Kappa Index (K), for the top, middle, and bottom sections of the index leaf and for the same sections in an older leaf from maize plants at the V4, V6, and V8 stages, under four levels of Mg in nutrient solution in greenhouse.

Leaf section	V4						V6						V8					
	VFD		VFDCA		GW		VFD		VFDCA		GW		VFD		VFDCA		GW	
	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K
Index leaf																		
Top	32.0	0.5	47.0	0.7	38.0	0.6	26.0	0.5	53.0	0.7	35.0	0.6	37.5	0.6	52.5	0.7	56.0	0.8
Bottom	35.5	0.6	49.5	0.7	42.0	0.7	38.5	0.6	57.0	0.8	45.0	0.6	39.5	0.6	58.0	0.8	63.0	0.8
Middle	32.5	0.6	47.0	0.7	50.0	0.7	39.5	0.6	46.0	0.7	46.0	0.7	40.5	0.6	56.5	0.8	57.5	0.8
Old leaf																		
Top	36.5	0.6	48.0	0.7	46.0	0.7	51.5	0.7	53.0	0.8	53.5	0.7	35.0	0.6	49.0	0.7	49.0	0.7
Bottom	32.5	0.6	42.5	0.7	44.5	0.7	47.5	0.7	53.0	0.8	49.5	0.7	38.5	0.6	52.5	0.7	55.0	0.7
Middle	34.5	0.6	48.0	0.7	41.5	0.7	49.0	0.7	56.5	0.8	48.0	0.7	34.5	0.6	47.5	0.7	45.0	0.7

Table 2. Global Percentage of Rights (GPR) of colors images using the volumetric fractal dimension (VFD), volumetric fractal dimension with canonical analysis (VFDCA), and Gabor wavelets (GW) to assess leaf Mg, and corresponding Kappa Index (K), for the top, middle, and bottom sections of the index leaf and for the same sections in an older leaf from maize plants at the V4, V6, and V8 stages, under four levels of Mg in nutrient solution in greenhouse.

Leaf section	V4						V6						V8					
	VFD		VFDCA		GW		VFD		VFDCA		GW		VFD		VFDCA		GW	
	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K	GPR	K
Index leaf																		
Top	47.5	0.7	57.0	0.8	57.0	0.8	57.5	0.8	49.5	0.7	49.5	0.7	53.0	0.7	53.5	0.7	48.5	0.7
Bottom	57.5	0.8	67.5	0.9	58.5	0.8	53.0	0.8	61.0	0.8	57.0	0.8	54.5	0.7	56.5	0.7	63.0	0.8
Middle	64.0	.08	75.5	0.9	66.0	0.8	55.0	0.8	57.5	0.8	54.0	0.7	57.0	0.7	54.0	0.7	61.5	0.8
Old leaf																		
Top	44.0	0.6	46.5	0.7	63.5	0.8	50.5	0.7	43.5	0.7	59.0	0.8	51.5	0.7	58.5	0.7	57.0	0.8
Bottom	48.5	0.7	58.5	0.8	60.5	0.8	57.0	0.7	57.5	0.8	55.0	0.7	53.5	0.7	57.0	0.7	64.5	0.8
Middle	48.5	0.8	55.0	0.8	55.5	0.8	53.5	0.7	50.5	0.7	57.0	0.7	48.0	0.7	55.0	0.7	56.5	0.8

Table 3. Confusion matrix by the volumetric fractal dimension with canonical analysis (VFDCA) of the middle of the index leaf (IL) of maize at stage V4 with Mg subjected to levels of Mg deficiency in the greenhouse.

Correct classification	% rights of VFDCA to the middle of the IL			
	0 mM	0.65 mM	1.3 mM	2 mM
	%			
0 mM	100	0	0	0
0.65 mM	2	82	10	6
1.3 mM	0	8	58	34
2 mM	0	14	24	62

Mg concentration are very similar to those for 0.65 mM and 2.0 mM Mg concentrations (Fig. 4a). However, the system was able to identify a reasonable amount of images.

The Mg concentration in IL at stage V4 grown in 1.3 mM Mg was 1.75 g kg^{-1} Mg (Fig. 4a) and very close to those grown in 0.65 mM and 2.0 mM Mg, showing 1.40 and 2.08 g kg^{-1} Mg (Fig. 4a), respectively, which may have hindered the identification of nutritional status of plants at AVS.

These results show that it is possible to obtain a good separation of images for plants in the greenhouse grown at 0.0 mM from those grown at 0.65 mM Mg, which are severe and moderate levels of Mg deficiency, respectively. This reasoning is supported by the Mg concentration in IL of plants at stage V4 (Fig. 4a) in which plants grown in 0.0 mM Mg solution had 0.68 g kg^{-1} Mg and those grown in 0.65 mM Mg solution had 1.40 g kg^{-1} Mg. Such difference allowed a better identification through the AVS.

CONCLUSIONS

The Mg concentration in solution had a quadratic response for Mg concentration in leaves (showing typical deficiency symptoms in case of undersupply) and therefore a quadratic response for shoot and root dry mass.

The AVS was able to identify the levels of induced Mg deficiency in maize plants conducted in a greenhouse. The middle section of the index leaf (IL) at the V4 stage was the best to evaluate the Mg deficiency in greenhouse-grown maize plants, considered trustworthy through the Kappa index (K = 0.9). The analysis of color images scored higher than grayscale images in all stages of development of the plant.

The AVS developed in this study identified the images of the leaves of maize, conducted in a greenhouse at the V4 stage with 75.5% rights using the middle section of the IL by the VFDCA technique, based on color images in V4 stage.

Acknowledgments

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