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Use of artificial vision techniques for diagnostic of nitrogen nutritional status in maize plants

L.M. Romualdo[a,*], P.H.C. Luz[a], F.F.S. Devechio[a], M.A. Marin[a], A.M.G. Zúñiga[b], O.M. Bruno[b], V.R. Herling[a]

[a] Department of Animal Science, College of Animal Science and Food Engineering, University of São Paulo (FZEA/USP), Duque de Caxias Norte Street, 225, Post Box 23, 13630-000 Pirassununga, SP, Brazil

[b] Department of Physics, Institute of Physics of São Carlos, University of São Paulo (IFSC/USP), Trabalhador são-carlense Avenue, 400, Poste Box 369, 13560-970 São Carlos, SP, Brazil

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ABSTRACT

The identification of the nutritional status of maize by foliar chemical analysis requires sampling of leaves when the plant is in an advanced stage of development, hindering corrective action in ongoing cultivation, if deficiency detection of a specific nutrient occurs. An artificial vision system (AVS) is a set of methods used for analysis and interpretation of images. Therefore, an AVS is being developed to identify nutrient deficiencies at different stages of plant development, especially in the early stages of growth, which may contribute to early diagnosis and correction in the same cycle of growth. The objective was to evaluate methods of digital image processing to develop the AVS to diagnose induced nitrogen deficiency in maize leaves. The experiment was done in greenhouse and the treatments were N doses (0.0; 3.0; 6.0 e 15.0 mMol L^{-1}) combined with three growing stages (V4, V7 and R1). The images of maize leaves were digitized in 1200 dpi. After scanning, leaves were chemically analyzed for N content and was determined the dry mass of plants. The studied methods in AVS were: Volumetric Fractal Dimension (VFD), Gabor Wavelet (GW) and VFD with canonical analysis. The omission and reduction of nitrogen in maize plants resulted in typical symptoms of N deficiency. The AVS was able to identify levels of nitrogen deficiency in the early stages of development of corn, with global percentage of right of 82.5% at V4 and 87.5% at V7. The GW technique with color images resulted in the better method for features extraction.

1. Introduction

The improvement of methods for identification of the nutritional status of plants, combined with the need for improvements in the efficient use of nutrients present in soil or applied through fertilization to obtaining high yields by crops, have led to search for new technologies, of the viewpoints agronomic, economic, environmental or operational. Mineral nutrients have essential role in plant metabolism in such a way that in cases where one of the nutrients is not in adequate amount the whole metabolism is prejudiced. Such disturbs usually can be identified by symptoms in plant development, such as chlorosis and necrosis, reduced growth and similar anomalies (Malavolta, 2006).

The Nitrogen (N) deficiency symptoms begin as leaf chlorosis, from tip to base as an inverted "V" pattern; dryness from the tip of the old leaves towards the central nervure; necrosis followed by leaf tearing, and thinning of stalks (Taiz and Zeiger, 2006; Marschner, 2012). Nitrogen is a constituent of all amino acids, amides, proteins, nucleic acids, nucleotides, polyamides and cytochromes, and is part of chlorophyll molecule (Malavolta, 2006). Lack of N delay cell division at the growing gems, decreasing leaf area and plant size, with prejudice of grain yield (Coelho, 2007).

The evaluation of nutritional state of the plants is usually done through chemical analysis or visual evaluation. Leaf chemical analysis imply sampling at certain phenological stage, which in practice does not allow to take remediation actions for the crop being cultivated. Visual diagnose is subjected to errors in interpretation because visual symptoms can occur simultaneously and also be confounded with pest attack or disease. Therefore, visual diagnose depends strongly on the operator's experience. The use of biometric measurements of leaves, related to the shape, area, texture and ribs, among others, provide quantitative, objective and accurate information that can be employed in the study of plant nutrition.

* Corresponding author. Address: Sorocaba Street, 985, 14075-130 Ribeirão Preto, SP, Brazil. Tel.: +55 19 35654267.

E-mail addresses: lilianeromualdo@yahoo.com.br (L.M. Romualdo), phcerluz@usp.br (P.H.C. Luz), ferdefatima@hotmail.com (F.F.S. Devechio), mario.marin@timacagro.com.br (M.A. Marin), agomez423@gmail.com (A.M.G. Zúñiga), bruno@ifsc.usp.br (O.M. Bruno), vrherlin@usp.br (V.R. Herling).

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In this context, computational models of artificial vision and mathematical models can contribute to a more detailed analysis of foliar structures through benchmark techniques and information extraction (Backes and Bruno, 2008).

An artificial vision system or computational vision may be defined as a set of methods and techniques through computer systems, which are able to interpret an image automatically or semi-automatically (Punam and Udupa, 2001). And are constituted in the steps: acquisition, image segmentation, feature extraction and classification/identification (Bruno, 2000; Gonzales and Woods, 1993). In literature there are several methods of taxonomies for image segmentation. However, the most traditional is adopted by Gonzales and Woods (1993), which defines three categories of segmentation: thresholding, based segmentation on edges and based on regions. Other methods are based on colors (Lim and Lee, 1990; Gonzalez et al., 1990; Moreira, 1999), the ones using neural networks and fuzzy logic (Bezdek et al., 1999) and those based on genetic algorithms (Ankenbrandt et al., 1990). Chromatic analysis is considered the information concerning the color. Among the methods used in this category of segmentation, can highlight the analysis of chromatic histograms (Cheng et al., 2001), chromatic moments (Stricker and Orengo, 1995) and set of colors on mappings (Cheng et al., 2001). The segmentation process selects regions for images analysis. The feature extraction step consists of methods to assess the regions of the images for their characterization. This step, the texture analysis considers the distribution and organization of pixels in a certain image region. In this context, methods that use measures like fractal dimension and lacunarity can measure the complexity of images, and generate digital signatures to characterize the image (Backes and Bruno, 2008).

The application and use of image analysis is utilized in agriculture for some time now, mostly in precision agriculture. Yang et al. (2000) applied artificial neural networks (ANN), trained with the back-propagation algorithm, to the development of a model capable of distinguishing young maize plants of weeds plants. Burks et al. (2005) used the same algorithm to study the recognition of weeds plants. Baesso et al. (2007) assessed the use of spectral indices, taken from digital images, to discriminate different doses of nitrogen in common bean. Sena Júnior et al. (2008) identified through image analysis nutritional stages of wheat plants. Silva et al. (2014) evaluated different methods for feature extraction in images of maize leaves in the V4 stage, grown in greenhouse under nutritional deficiency induced of magnesium. With the obtained results, it was found global percentage of right 76% with reliable Kappa index. Silva Júnior et al. (2012) determined the percentage of vegetation cover of weeds plants in the crop beans, under the no-tillage and conventional, using digital image processing and geostatistics. Abrahão et al. (2013) conducted a study of classifiers based on different combinations of bands and spectral indices of original images to discriminate foliar nitrogen and chlorophyll, also in the crop beans.

Being so, the use of image processing in agriculture can be an auxiliary important tool in the soil and plant management. The present research was focused in the development of an AVS for N diagnosis in maize plants, for further validation in field. The objective of this study is to present an evaluation of methods of image processing used to identify and evaluate induced N deficiency in maize.

2. Material and methods

2.1. Experiment in greenhouse

The experiment was done in a greenhouse of the Animal Science Department of the College of Animal Science and Engineering Food (FZEA/USP) at the Pirassununga-SP campus. The crop tested was maize (*Zea mays* L.), hybrid DKB 390®, grown in a hydroponic system in nutrient solution.

Experimental design was fully random, in a 4 × 3 factorial with four replications. The factors established for the study consisted of four levels of N concentration in nutrient solution: T1 = complete omission (0 mMol L^{-1}), T2 = 20% of full level (3.0 mMol L^{-1}), T3 = 40% of full level (6.0 mMol L^{-1}) and T4 = full level (15 mMol L^{-1}), combined in three time sampling of collecting leaves (phenological stage), when plants were with four fully expanded leaves (V4), seven leaves (V7) and silking (R1), totaling 48 experimental units (4 level of N × 3 sampling stage × 4 replications). The concentration of elements in nutritional solutions was determined after a previous trial having the Hoagland and Arnon (1950) solution as reference.

Seeding was done in plastic trays filled with washed sand until two weeks after emergence. After that, two plants were transferred to 3.5 L pots (experimental units) and supported by a piece of foam and maintained there until stage V7, when they were transferred to 10 L pots until stage R1. After transplanting, plants were kept in a 50% diluted nutrient solution for them to adapt, during 5 days. Nutrient solutions were replaced 15 days after transplanting, and henceforward weekly. When needed, pot solution was leveled with deionized water and pH adjusted between 5.0 and 6.0 with HCl 1 N and NaOH 1 N. Each pot had air bobbling during 10 s at each 30 s interval.

In each collecting times established, 16 pots (experimental units) were sampled (sample destructive). Sampled material was split in (a) above ground portion (shoot, Sh); (b) root (Rh); (c) index leaf (IL) of the growing stage (V4 = leaf 4; V7 = leaf 7, and R1 = opposite leaf below the first ear) and (d) old leaf (OL). At all sampling times (V4, V7 and R1), the OL collected in the corn plant corresponded to older leaf that was not senescent.

The shoots, index leaves and old leaves after collection of images, were rinsed in deionized water. The roots were washed in running water and then were washed in the following order: water with a neutral detergent, deionized water, hydrochloric acid (HCl) diluted in deionized water and finally deionized water. Posteriorly, the plant parts were dried in an oven with forced air circulation at a temperature of 65 °C, for approximately 72 h, to determine the dry mass and then grind with 2 mm screen and saved in plastic bags for further analyses of N determination according to methodology described in Bataglia et al. (1983).

2.2. Statistical analysis for dry mass and N concentration

Statistical analysis model below was accomplished for the dry mass yield and accumulation of nitrogen in shoot and root of maize plants, and N concentration in index leaves. Results were statistically processed using variance analysis. According to Steel et al. (1997) the statistical model used was as follows

$$\text{Yijk} = m + \text{Ei} + \text{Nj} + \text{ENij} + \text{eijk} \quad (1)$$

In the model, Yijk is the observed value at parcel that received ij treatment on repetition k; *m* is the average; Ei is the effect of the development stage of maize; Nj is the effect of nitrogen levels at parcel; eijk is the effect of uncontrolled factors at parcel that received ij on repetition k. When the *F* test was significant ($P \leqslant 0.05$) only for Nj, has been done only one polynomial regression analysis for all stages. When the *F* test was significant ($P \leqslant 0.05$) for ENij, in the other words, when there was interaction between the levels of N applied and the developmental stages, the splitting aimed to study the levels within of the Ei for the N content in the index leaves. And for the accumulated N in different plant parts were studied developmental stages within each level of N (Nj) in the nutrient solution.