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Abstract

The measurement of nutrient status using leaf tissue analysis requires sampling acquisition at the mature stage of plant development. The artificial vision system (AVS) is a set of methods for image interpretation which can be potentially used to identify nutrient deficiency in plants during the first stage of plant development, allowing early nutrient status correction. This study had the objective to grow potassium (K) deficient maize plants (*Zea mays L.*) and test AVS routines. The experiment was done in a greenhouse at São Paulo University campus of Pirassununga, Brazil. Plants were grown in a hydroponic system with 0, 47, 94 and 234 mg L-1 of K, with four replications. Old leaves and number 4 leaves (leaves typical of V4 development stage of maize) were sampled, scanned with 9600 dpi resolution and chemically analyzed. The AVS methods tested were: Volumetric Fractal Dimension (VFD), Gabor Wavelet (GW) and VFD with canonical analysis (VDFCA). The images of the bottom, middle and top of each leaf, both in gray and color scales were submitted to these methods. The increase in K concentration in the nutrient solution increased the K levels in leaves which resulted in appearance of the typical K deficiency symptoms in the plants grown in the absence or low level of this nutrient. In old leaves, the GW method applied to images from the bottom of the leaf in gray scale was the most precise, reaching 79% of right diagnoses. The AVS was capable of diagnose K levels in maize leaves.

Keywords: mineral nutrition, *Zea mays* L., visual diagnosis, artificial intelligence.

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INTRODUCTION

Mineral nutrients have specific and essential functions in plant metabolism. In case the nutrient is not available in adequate supply to the plant, the cell metabolism is depressed. Changes in metabolism are usually revealed through visual symptoms such as leaf yellowing, leaf necrosis, and decrease in growing, among others. Potassium activates about 60 enzymes involved in biochemical routes such as sugar transport, stomata control, osmotic control and photosynthesis (Malavolta, 2006).

The evaluation of the nutritional status of plants is usually done by chemical analysis or by visual interpretation. Visual interpretation is particularly propitious to failure, since it can be confused with symptoms of deficiency of other nutrient and /or to the presence of plant pests and diseases (Oliveira et al., 2007). Therefore the visual interpretation has a great influence of the observer experience. The chemical analysis of leaves requires sampling at specific phenological stages of plant development, which makes it unsuitable for nutrient management of annual crops.

In such scenario, the development of new technologies that allow increase in crop production by providing precise, fast and non-subjective information would be an excellent tool to nutrition management of crops, allowing adequate nutrient supply early enough to impact the crop in the same season.

The Artificial Vision System (AVS) is a set of computational methods and techniques capable to interpret images (Punam & Udupa, 2001). Through the development of intelligent herbaria, it would be possible to diagnose early nutrient deficiency symptoms and provide action that would impact crop production in that very same plant cycle. Each leaf deficiency results in a characteristic symptom such as change in pigmentation, necrosis, decreased growth, change in surface texture, among others. The AVS may identify each one of these symptoms and relate it to a specific nutrient deficiency.

The objective of this study was to evaluate the digital image processing methods of AVS applied to the K deficiency in maize plants (*Zea mays* L.) and validate it by comparing with leaf chemical analysis.

MATERIAL & METHOD

The experiment was done in the Animal Science and Food Engineering College (FZEA) of University of São Paulo (USP) at the Pirassununga-SP campus. The crop tested was maize (*Zea mays* L.), hybrid DKB 390, grown in a hydroponic system in a greenhouse. Nutrient supply was done through Hoagland & Arnon (1950) nutrient solution.

Four doses of K were used: 0 mg L-1 K (T1); 47 mg L-1 K (T2, 1/5 of T4); 94 mg L-1 K (T3, 2/5 of T4) and 235 mg L-1 K (T4). Sampling for K determination were done at: 1) V4 maize stage (24 days after plant emergence);

2) V7 (44 days after plant emergence); and 3) R1 (78 days after plant emergence).

Experimental design was fully random in a 4x3 factorial (4 K levels and 3 sampling events) with four replications. Since leaf chemical analysis is destructive, 16 experimental units (pots) were subtracted at each sampling event.

Sampled material was split in a) above ground portion (shoot); b) root; 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). Dry mass and K concentration in tissues were determined in these materials.

Image acquisition for the AVS analysis were done in a scanner with 9,600 dpi (dots per inch) attached to a Personal Computer (PC). The images processed in the AVS system were those from V4 maize stage. Images from the leaf 4 (IL) and oldest leaf (OL), which is the first leaf to show K deficiency symptoms.

The work to better adjust the SVA methods for K deficiency detection was done at the Physics Institute of USP at São Carlos campus (IFSC – USP). Acquired images were segmented (regions of the image were selected for analysis), characterized by extraction methods (routines capable of select regions of the images for characterization) and classification/identification.

Three sectors of each scanned leaf were used: bottom, middle and top, both for gray and color scale images. Twelve windows were collect from each leaf image (6 at each side of the central nervure). Windows with noise or defects were discarded. The AVS techniques evaluated were: Volumetric Fractal Dimension (VFD), Gabor Wavelet (GW) and VFD with canonical analysis (VDFCA).

Statistical analysis

Results were subjected to variance analysis according to experimental design described above. In cases with significant F, simple or multiple polynomial regression analysis was done, for the main effect (K dose) or secondary (sampling event), since the variables were quantitative.

RESULTS & DISCUSSION

The Figure 1 shows the accumulation of K in shoot (a) and root (b) of maize plants during their growth cycle and the Figure 2 in IL (a) and OL (b). It can be seen an increase in K content in root, shoot, IL and OL as the plants grew older, particularly in those plants with sufficient K supply (Figures 1 and 2).

There was a gradual and significant increase ($P<0.01$) in K accumulation in the plant tissues analyzed as the K content in nutritive solution increased. As expected, plants with no K supply had small content of K in all tissues analyzed (Figure 1 and 2). Such behavior assures that the symptoms observed were related to K deficiency. It was not possible to analyze the K content in OL leaves at 24 days because the small amount of material available was insufficient to proceed the analysis. Therefore, Figure 2 shows only the K content at 44 and 78 days (stages V7 and R1).

The greatest K accumulation in the leaves was observed at 61 days after maize emergence in plant supplied with complete K dose (Figure 2a), surpassing the values observed in OL. This is in agreement with the high K mobility in plants, that is, redistribution from OL to IL (from old leaves towards new leaves).

The potassium is the second most absorbed nutrient by plants (after nitrogen), being maximum absorption during the vegetative plant development (30 to 40 days), suggesting K as an important nutrient for "starting" plant development (Gamboa, 1980).

According to Malavolta (2006), K is the enzymatic activator of more the half hundred enzymes involved in biochemical routes, stomata functioning, osmotic regulation and photosynthesis. Potassium is the most important inorganic solute in plants osmotically active, in the view of Mengel & Kirby (1980), being essential to cell growth.

The number of images correctly classified by the VFD, GW and VFDCA procedures is in the Table 1, for both gray and color scales, at the V4 maize stage. It can be observed that the best result (79.0%) was obtained using the bottom of OL using the GW method, in gray scale images.

In Table 1 it also can be observed that for the GW technique, the AVS had the greatest efficacy at the FV, since correct diagnostics were performed in more than 70% of cases, suggesting that old leaves are the best to identify K deficiency in maize. This can be explained by the K mobility in plants, since the element moves from old tissues towards the new ones. Malavolta (2006) reports that K deficient plants had firstly yellowing followed by tanning at the borders of old leaves, and such symptoms move gradually towards the central nervure.

The confusion matrix of images from the bottom of FV processed by the GW routine and images in gray scale of V4 maize plants at K supply levels is shown in Figure 3. The confusion matrix shows how many image windows were correctly classified by this technique among the total 50 analyzed.

At the confusion matrix in Figure 3, images correctly classified were 49 out of 50 in T1, 29 images out of 50 in T2, 39 out of 50 in T3 and 41 out 50 in T4. Four images from T3 were wrongly classified as T4 and 7 as T2. There was no image error classification between T3 and T1 (zero).

It should be highlighted that one of the advantages of the AVS is to capture symptoms of nutritional deficiency in intermediate stages, which are difficult to be perceived by the human eye. In this sense, the GW routine in gray scale reached 58% of rights (100x29/50) for T2 and 78% (100x39/50) for T3 (Figure 3), which can be considered good results since the biological symptoms were moderate.

CONCLUSION

The Artificial Vision System (AVS) was efficient for identification of K levels in maize leaves, being the best routine the Gabor Wavelet (GW) applied to bottom portion of old leaves in gray scale images at the V4 stage of plant development, reaching 79% of correct diagnostics.

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Figure 1. Potassium accumulation in shoot (a) and roots(b) of maize plants during the growing cycle as a function of increasing levels of K in the nutritive solution.

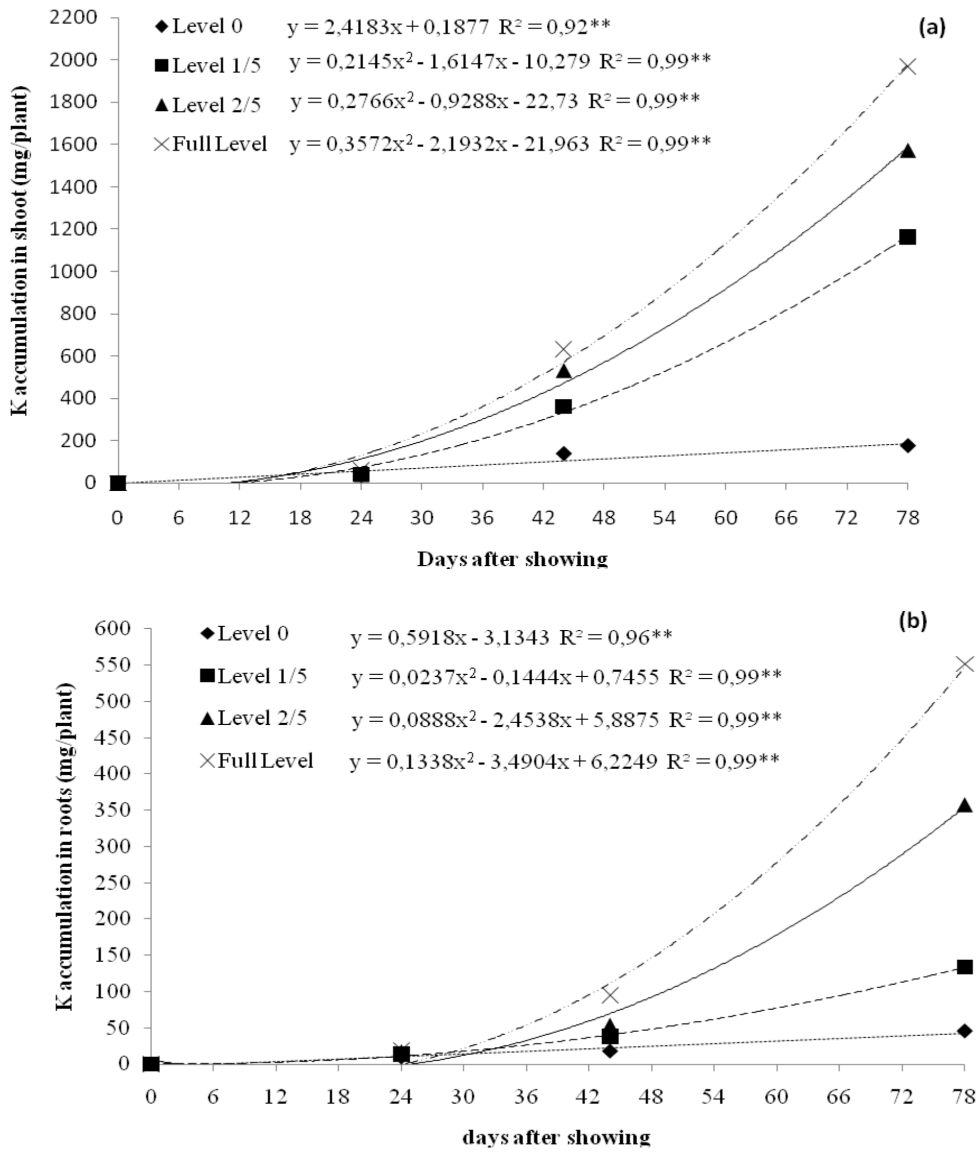

Figure 2. Potassium accumulation in index leaves (a) and old leaves (b) of maize plants during the growing cycle as a function of increasing levels of K in the nutritive solution.

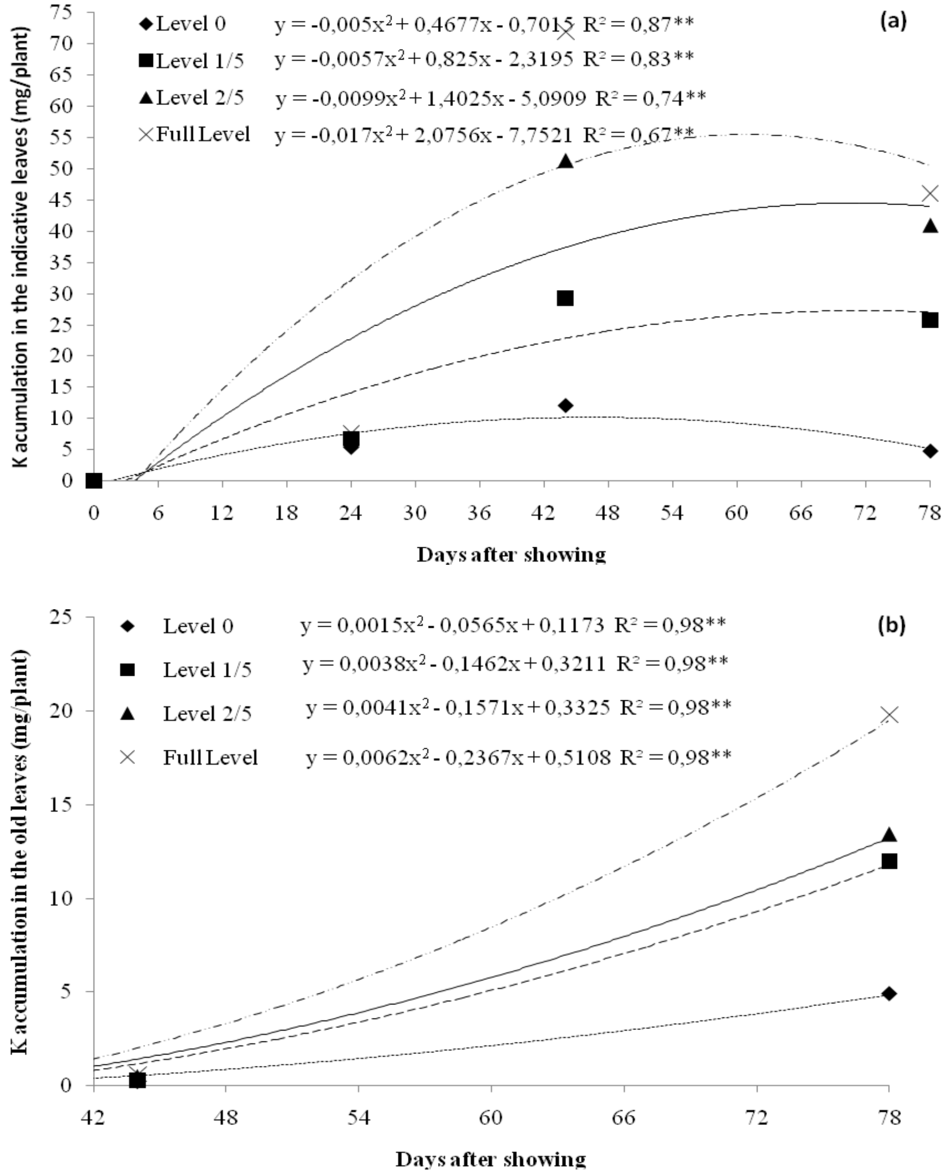

Table 1. Number of Gray and color images correctly classified by the VFD, GW e VFDCA routines in maize leaves at the V4 stage as a function of K defficiency.

Number of images with correct diagnostic			
Routine	**DFD**	**GW**	**DFDCA**
	Gray Scale Images		
Top IL	37.00	47.50	37.50
Bottom IL	42.00	51.00	51.00
Middle IL	33.50	54.00	54.00
Top OL	63.50	77.00	71.50
Bottom OL	60.00	79.00	76.50
Middle OL	58.50	73.50	69.00
	Color Scale Images		
Top IL	37.50	63.50	45.00
Bottom IL	32.00	64.50	43.50
Middle IL	40.00	63.50	42.00
Top OL	67.00	65.00	69.50
Bottom OL	63.00	59.50	61.00
Middle OL	66.00	41.00	70.00

Volumetric Fractal Dimension (VFD), Gabor Wavelet (GW) and VFD with canonical analysis (VDFCA)

Figure 3. Confusion Matrix of Gray scale images from bottom portion of old leaves (OL) of maize plants at the V4 stage submitted to K deficiency, using the GW routine.

T1	**T2**	**T3**	**T4**	
49	0	1	0	**T1**
0	29	9	12	**T2**
0	7	39	4	**T3**
0	9	0	41	**T4**