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Full Length Research Paper

Nitrogen determination on tomato (*Lycopersicon esculentum* Mill.) seedlings by color image analysis (RGB)

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In order to investigate the effectiveness of a new method based on color image analysis and the Minolta SPAD-502 chlorophyll meter for the diagnosis of nitrogen deficiencies of tomato seedlings, a field experiment was conducted. In this study, five levels of nitrogen fertilization were established so as to induce nitrogen deficiencies in tomato seedlings. Thirty-five days after sowing, total nitrogen was evaluated by laboratory analysis. The chlorophyll index was determined using a SPAD-502 chlorophyll meter. Also, color images were taken with a digital camera; the color images were processed in MATLAB in order to determine the averages of the red color, green color and the blue color. The relationships between variables were analyzed by linear regressions and a one way analysis of variance ($p < 0.01$). Results showed that color image analysis correlated better with the status of plant nitrogen than the SPAD. From the color image analysis, the red and blue colors were more accurate predictors of nitrogen status on plants with R^2 above 0.89. Color image analysis provides an accurate and quick way for nitrogen estimation and can contribute for early detection of nitrogen deficiency in tomato seedlings. The SPAD method is not a reliable way to estimate the nitrogen status on tomato seedlings.

Key words: Color image analysis (RGB), chlorophyll meter, nitrogen deficiency.

INTRODUCTION

The overall goal of precision agriculture is to make cultural operations more efficient, reduce environmental impact while enhancing crop quality and yields. The diagnosis of symptoms caused by nutrient deficiencies in plants is important in precision agriculture; this requires accurate and reliable techniques that permit the identification of physiological disorders with opportunity. Most of these physiological disorders affect the composition and proportion of pigments on leaf tissue (Bacci et al., 1998). Tomato is an important vegetable crop in which nitrogen (N) has a major influence on productivity levels (Badr and El-Yazied, 2007). The objective of N management consists of supplying enough N to achieve maximum crop yields (Amanullah, 2010). Efficient N management in tomato production can be attained by using suitable evaluations of the plant's nutritional status. Alternatively, quick, non-destructive procedures have been proposed to evaluate tomato N status, correlations between N status and chlorophyll or polyphenolics content on leaves

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Abbreviations: **SPAD**, Soil plant analysis development; **RC**, red color; **GC**, green color; **BC**, blue color; **EDTA**, ethylene diamine tetra acetic acid, **N;** nitrogen.

(Sexton and Carrol, 2002; Wang et al., 2004; Cartelat et al., 2005), and leaf greenness determination by the Minolta SPAD meter (Finnan et al., 1997, Richardson et al., 2002; Chang and Robison, 2003). Kawashima and Nakatani (1998) presented a method to determine the chlorophyll content on plants using a video camera and a computer. Recently, digital photography has been used for detecting the level of N fertilization (Jia et al., 2004). These authors employed aerial photographs in order to estimate the level of N fertilization in a wheat field. They found a positive correlation between the image analysis and the concentration of N in the field. Wiwart et al. (2009) analyzed changes in the color of three different species of plants that were subjected to N, phosphorous, potassium and magnesium deficiencies. This was done by carrying out digital color image analysis using the Hue, Saturation, Intensity (HSI) model for color image processing. They found that the Euclidean distances between the colors of leaves at successive nodes, makes it possible to accurately determine the responses of the analyzed species to deficiencies of the four basic aforementioned macro-nutrients. The objective of this research was to determine the nitrogen deficiencies on tomato (*Lycopersicon esculentum* Mill.) seedlings by utilizing two indirect methods: Digital color image analysis based in Red, Green and Blue (RGB) color space and the chlorophyll content in leaves.

MATERIALS AND METHODS

Experimental site and greenhouse

The experimental site is located in Queretaro State University, campus Amazcala, Queretaro, Mexico at a longitude of 100° 16' W; latitude, 20° 42' N; altitude, 1920 m.

The greenhouse was a gothic single span greenhouse type with an area of 56 m^2 area (7 × 8 m) with only lateral ventilation (24 m^2). The cladding material was a single layer of long term polyethylene plastic with a shading net on top of the plastic to protect against high temperatures.

Biological material and containers

The tomato seeds were of indeterminate growing. The substrate was a commercial organic material with 1.23% total N and $N\text{-}NO^-_3$ 250 mg/kg.

The germination container for the plants was made of polystyrene with 200 conic cavities. Each cavity held 25 ml of material. The dimensions of the containers were width 36 cm, length 62 cm and height 7 cm.

Management of sowing and emerging

First, all the containers were disinfected using an organic solution based on citrus seeds extract. The concentration used to prepare the disinfectant solution was 8 ml of extract to 1 L of water. The tomato seeds were kept in water for one hour before sowing to accelerate the germination process. The organic substrate was watered to field capacity. Then the containers were filled with the organic substrate up to seven-eighths of their capacity in order to place a tomato seed (about 0.5 cm deep). These were then covered with vermiculite and watered. Finally, all containers were put one upon another in piles of seven containers and then covered with a layer of black plastic. All the containers were kept in a germination room at a temperature of about 25°C and 70% of relative humidity until the first plants emerged (about four days after been sown). When this occurred all containers were transferred to the plastic greenhouse where the plants were going to be grown. The sowing date was September 15th 2009.

Mineral nutrition

The basic nutrient solution used was the one presented by Steiner (1984). The water used to prepare the nutrient solution was rain water with the following characteristics: pH = 7.90; EC = 0.30 dS/m; and the ions in me/L: Ca^{2+} = 0.59, Mg^{2+} = 0.02, Na^+ = 0.05, K^+ = 0.07, CO_3 = 0.08, $HCO3^-$ = 1.63, SO_4^{2-} = 0.14 and $N\text{-}NO_3^-$ = 0.1 mg/kg.

In order to complete the nutrient solution the following substances were used: Calcium nitrate ($Ca(NO_3)_2$); Potassium nitrate (KNO_3), Phosphoric acid (H_3PO_4, 85%), Mono potassium phosphate (KH_2PO_4), Potassium sulfate (K_2SO_4), Magnesium sulfate ($MgSO_4$), Magnesium nitrate ($MgNO_3$), Boric acid (H_3BO_3), Iron chelate (Fe, 13%), Manganese chelate (Mn, 13%), Zinc chelate (Zn, 14%), and Copper chelate (Cu, 14%); the chelate was EDTA.

Five $N\text{-}NO^-_3$ rates in the nutrient solutions (N treatments): 0, 4, 8, 12 and 16 me/L were evaluated in order to induce different N status in the plants. The electric conductivity was kept constant. Table 1 shows the final nutrient solutions. The treatments were applied after two true leaves appeared. Prior to that point only water was used.

Experimental design

The experimental design adopted was a completely randomized design according to Snedecor and Cochran (1976), with six replications. The experimental unit consisted of 100 plants and the sampling size was only ten plants at random election. Five variables were measured: Chlorophyll index (CI), three color bands and total N content in plant were determined 35 days after sowing. All variables were taken from the same sampling group. Data were subjected to analysis of variance by the general linear models (GLM) procedure and means comparison by Tukey's test using SAS methods (1990). The relationships between total N content in plant as independent variable and chlorophyll index (CI) and the three color bands as dependent variables was analyzed by correlation according with Spiegel (1992) and Blanco (2001).

Chlorophyll measurements

A Minolta SPAD-502 chlorophyll meter was used to determine a CI. This measures absorption at 650 and 940 nm wavelengths to estimate chlorophyll levels. Leaves were removed and one measurement was taken immediately for each leaf and averaged to provide a single CI per plant. The SPAD sensor was randomly placed on leaf tissue (Pinkard et al., 2006). With the SPAD, chlorophyll content is calculated based on the amount of light transmitted by the leaf in two wavelengths where the light absorbed is different. The maximum of light absorbed occurs in regions of blue and red with little light been absorbed in the green region. For this reason, the wavelengths used for measurement, are those of red and infrared. The light emitted by the SPAD corresponds to red light (650 nm) and infrared light (940 nm). Light passes through the leaf and arrives at the receiver becoming an electrical signal which is amplified and digitized (Kieffer, 2009).

Table 1. Concentration of nutrients and electric conductivity in the five nutrient solutions.

Treatment	NO_3^{-2}	$H_2PO_4^-$	SO_4^{2-}	K^+	Ca^{2+}	Mg^{2+}	Fe	Cu	Mn	Zn	Bo	EC
	(me/L)						(mg/L)					(dS/m)
1	0.0	2.5	9.4	6.9	8.4	4.0	1.90	0.05	0.80	0.30	0.47	2.0
2	4.0	2.0	9.9	6.9	8.4	4.0	1.90	0.05	0.80	0.30	0.47	2.0
3	8.0	1.5	10.4	6.9	8.4	4.0	1.90	0.05	0.80	0.30	0.47	2.0
4	12.0	1.0	7.3	6.9	8.4	4.0	1.90	0.05	0.80	0.30	0.47	2.0
5	16.00	1.0	3.3	6.9	8.4	4.0	1.90	0.05	0.80	0.30	0.47	2.0

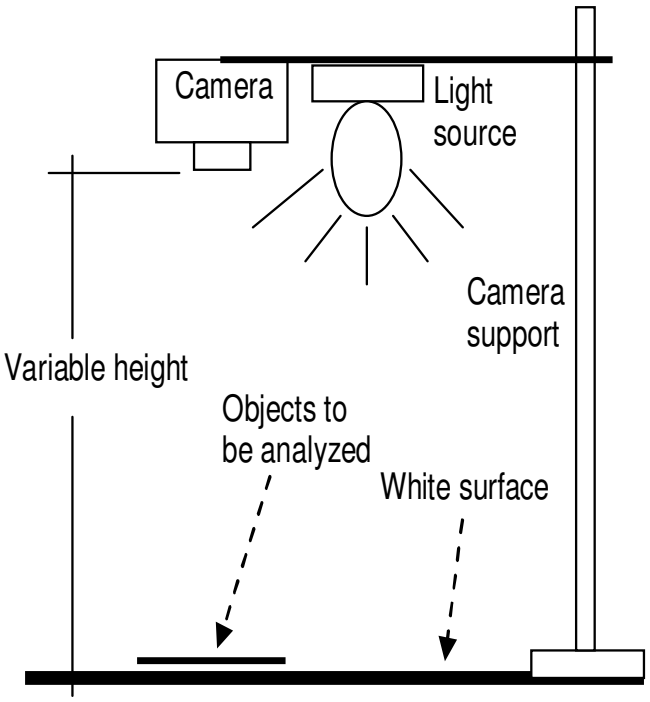

Figure 1. Camera set up.

Color images acquisition

All leaves from each plant were laid on a white background to ensure that the photographs would have only two colors with the highest contrast. It is also mandatory that no other objects appear in the photograph because they may be mistakenly included as part of leaves. For this study, all leaves were place with the upperside facing up wards. The camera used to acquire the images was a SONY cyber-shot model DSC-W 120 with resolution of 7.2 megapixels. No flash was used. All photographs were taken inside a dark room and so a white light source was used. In this case a fluorescent lamp of 100 watts bulb was used, all this in order to ensure the same light condition for all photographs (Figure 1).

Image analysis

The image analysis was done by determining the three band colors (RGB). For each image, a file in *.txt was generated; the file is three columns of values listed; red color (RC), green color (GC) and blue color (BC) from 0 - 255 of each pixel by means of sweeping the whole image. For each value list, three averages were determined and used to estimate their correlation with the N content in plant leaves. This analysis was performed using the tool-box for image analysis as used in the MATLAB V 7.1 which works within Windows platform. Table 2 presents the developed code of the used functions for the image processing.

N determination by laboratory

The samples were dried at 72°C for 48 h. The N content was determined using the Kjeldahl digestion technique (Bremner, 1986) to provide a standard method to calibrate the SPAD and the color image analysis methods. This method determines not only protein, and it is appropriate for determining N-total: N-organic (proteins and nucleic acids in their various states of degradation, such as amino acids, ureido, amines, amides), and even N-mineral as ammonium. With this method, all N-organic is converted to ammonium, then to ammonia and in this form is quantified.

RESULTS AND DISCUSSION

Effect of nitrogen applied to tomato on the different variables evaluated

The nitrogen applied to the tomato had a highly significant effect on all evaluated variables ($p < 0.01$). This situation was reflected in the comparison of means (Table 3).

Content of N in plants

The results observed in total N content in plants responding to different amounts of nitrogen applied to tomatoes, showed an expected quadratic trend. The trend of the curve also suggests a symmetrical and mesokurtic distribution (Snedecor and Cochran, 1976) under hipotetical test of higher levels of nitrogen (Figure 2). Similar results have been reported previously (Basoccu and Nicola, 1995; Balliu et al., 2007). On the other hand, these results reinforce the notion that this variable is a good indicator to assess the validity of the measurements with other methods.

SPAD readings

The results show that CI is proportional to concentration

Table 2. MATLAB code for color image analysis.

Code Line	MATLAB code lines
1	Clear all; % Remove items from the workspace.
2	A = imread ('photo.jpg'); % Reads the photo into A.
3	RL = 70; GL = 70; BL = 70; % Sets the values for image filtering.
4	Blacks = 0; % Sets the counter "blacks" equal to zero.
5	For i=1:2304 % Here initiates the process of filtering.
6	For j=1:3072.
7	If (A(i, j, 1) >= RL && A(i, j, 2) >= GL && A(i, j, 3) >= BL)
8	A(i,j,1) = 0; A(i,j,2) = 0; A(i,j,3) = 0;
9	Blacks = blacks+1;
10	End; end; end; % Ends the process of filtering.
11	Imshow (A);% Plots the filtered image.
12	R = double(A(:, :, 1)); G = double(A(:, :, 2)); B = double(A(:, :, 3)); % Writes the Red, Green and Blue colors into R, G, B arrays.
13	Red = 0; % Sets "Red" equal to zero.
14	For I = 1:2304% This cycle sums the Red value from each pixel.
15	For j = 1:3072.
16	Red = Red + R(i,j);
17	End; end;
18	Red = Red/((2304*3072)-blacks); % Gets the average of the Red value %. The code is repeated from line 13 to line 18 for the Green and Blue bands.
19	Red % Displays the Red average value in MATLAB Command window.
20	Green % Displays the Green average value in MATLAB Command window.
21	Blue % Displays the Blue average value in MATLAB Command window.

An explanation follows code lines.

Table 3. Means comparison by Tukey's test of the evaluated variables.

Treatment	N (g/kg)	Chlorophyll index	Red color	Green color	Blue color
T1	17.82a	20.17a	56.54a	60.38a	44.79a
T2	28.49b	35.23b	41.58b	52.38d	20.61b
T3	35.87c	36.16b	39.85c	56.85b	16.92c
T4	36.69c	37.19b	39.30c	56.88b	15.12d
T5	42.44d	39.21c	35.66d	54.26c	12.87e

Levels not connected by same letter are significantly different ($P<0.01$).

of N in the nutrient solution (Figure 3a). The correlation of N status on plants with CI obtained with a linear model is R^2 = 0.87 (Figure 3b). This is a good correlation and indicates that the tendency of N in plants and CI is similar, though the statistical analysis shows that CI does not indicate significant differences for treatments: Two, three and four making this technique not suitable for N estimation on tomato seedlings. Rostami et al. (2008) clearly indicate that factors other than N can influence growth, chlorophyll and N relationships and thus the interpretations of SPAD readings. In general, they found that the SPAD meter is not a good technique to predict N status in corn, though they obtained a R^2 = 0.84 for linear model. According to Sainz and Echeverria (1998), the chlorophyll in plats varies not only with N status on plants but with temperature, for this reason. The results evaluated with the SPAD are not reliable at all.

Image analysis

The results show that red colour (RC) values are inversely proportional to the N concentration in the nutrient solution (Figure 4a). The determination of N status on plants by the RC is well predicted by a linear model with R^2 = 0.91 (Figure 4b). As observed in Table 3,

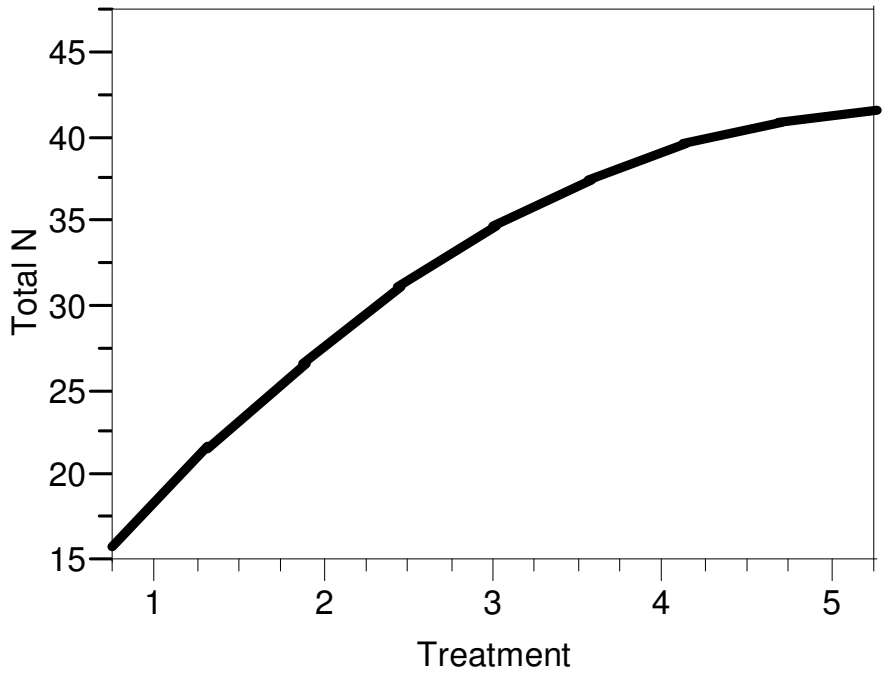

Figure 2. Total nitrogen concentration on plants versus N on nutrient solution.

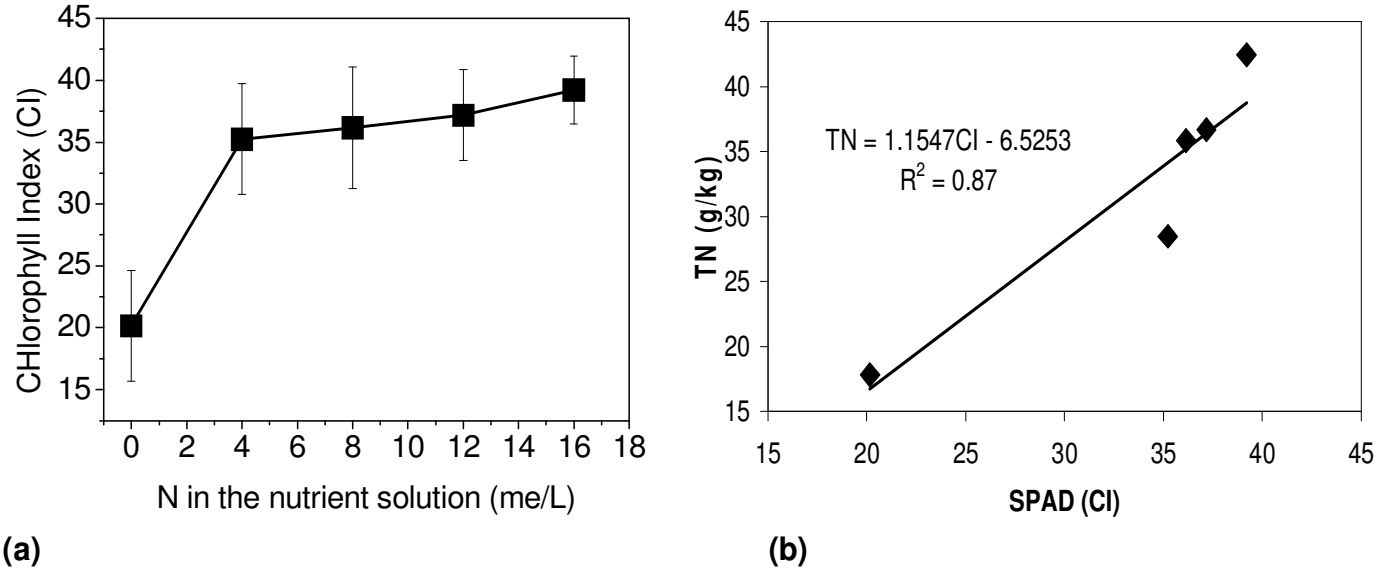

Figure 3. Chlorophyll index (CI).

only two levels of applied nitrogen had values statistically equal). This is also confirmed by the statistical analysis where the same behavior is shown for the N status on plants and the RC.

The green colour (GC) has no definite relation with the N concentration in the nutrient solution (Figure 4c). The prediction of N status on plants by the GC is predicted very poorly by a linear model with $R^2 = 0.28$ (Figure 4d). Though the statistical analysis shows the same behavior for the N status on plants, the correlation indicates that this color is not suitable for N determination on tomato seedlings.

The results also show that the blue colour (BC) values are inverse to the N concentration in the nutrient solution (Figure 4e). The determination of N status on plants by the BC is well predicted by a linear model with $R^2 = 0.89$ (Figure 4f).

As in the case of RC, BC values were a better option

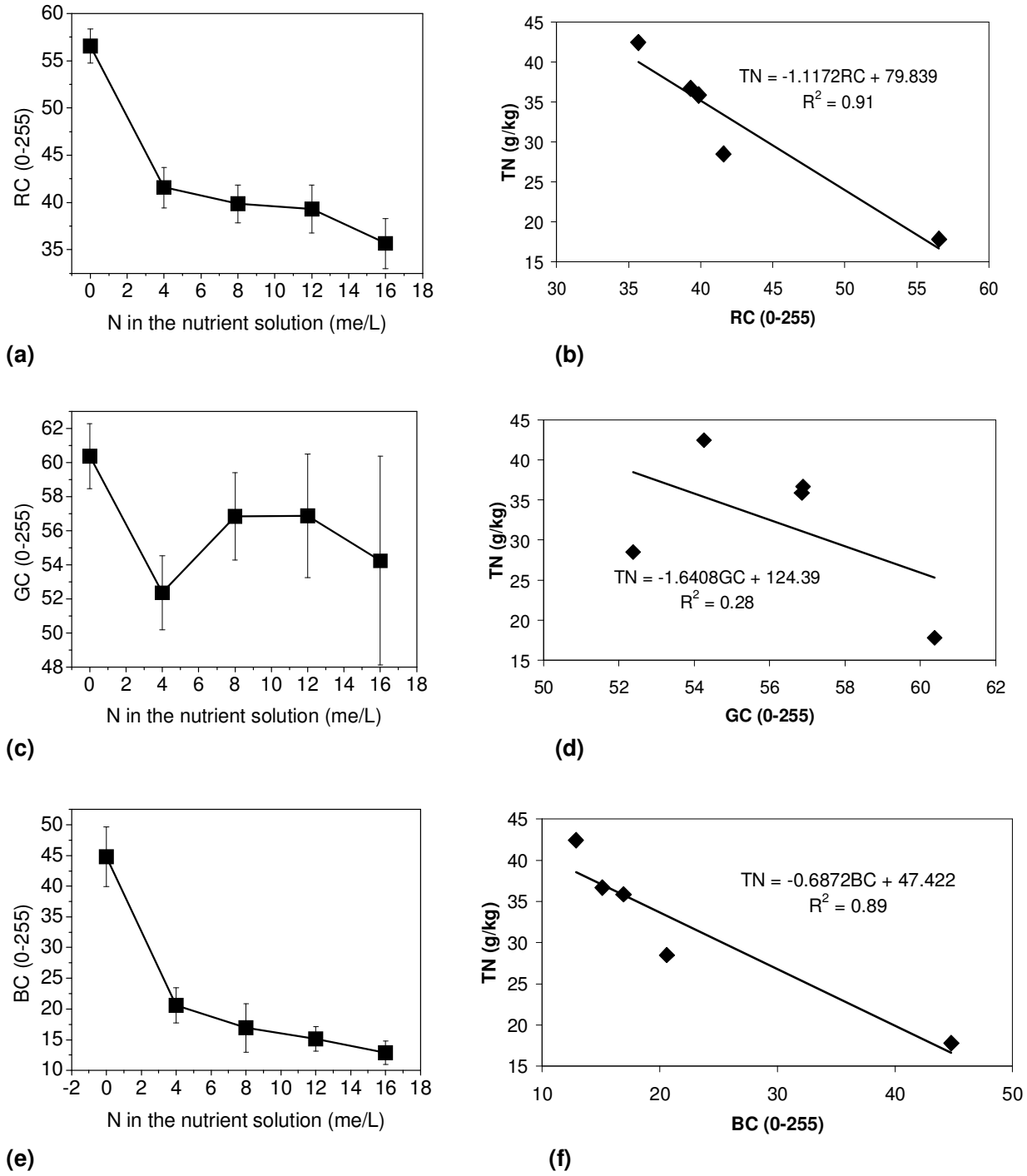

Figure 4. Image color analysis. (a) RC versus N in the nutrient solution, (b) Scatter plot TN versus RC, (c) GC versus N in the nutrient solution, (d) Scatter plot TN versus GC, (e) BC versus N in the nutrient solution; and (f) Scatter plot TN versus BC. T = Treatment; CI = chlorophyll index; RC = red color; GC = green color; and BC = blue color.

than the SPAD to determine the total nitrogen in tomato plants. Although the correlation coefficient was slightly minor than the case of RC, the effect of nitrogen application was statistically different at each level and with the same trend (Table 3).

These results indicate that the red and blue bands carry the most important information to identify N deficiencies on tomato seedlings.

Pagola et al. (2009) proposed a greenness index using RGB components of a color image; they calculated the

correlation between the index and measurements obtained with a SPAD-502 chlorophyll meter. They reported a better or equal prediction for the greenness index than the SPAD measurements. On the other hand, Kawashima and Nakatani (1998) reported an index using only the red and blue color bands [(red - blue)/(red + blue)] as the most applicable function to estimate the chlorophyll content on plants. These results are in line with the results of the current study.

Similarities between images color analysis and the SPAD are because both methods use the same principle to determine the intensity of green color of the leaf tissues. The intensity of greenness in tomato seedlings depends primarily on the content of chlorophyll, which is closely related to N content (Gandrup et al., 2004).

Conclusion

The results of the current study suggest that color image analysis can be applied to estimate the N status on tomato seedlings using RC and BC. From the color image analysis, RC and BC are the most accurate predictors of N status on plants with R^2 above 0.89. Color image analysis provides an accurate and quick way for N estimation and can contribute for early detection of N deficiency on tomato seedlings. The SPAD method is not a reliable way to estimate the N status on tomato seedlings.

REFERENCES

Amanullah SP (2010). Timing and rate of nitrogen application influence grain quality and yield in maize planted at high and low densities. J. Sci. Food Agric. 90(1): 21-29.

Bacci L, De Vincenzi M, Rapi B, Arca B, Benincasa F (1998). Two methods for the analysis of colorimetric components applied to plant stress monitoring. Comput. Electron. Agric. 19(2): 167-186.

Badr M, El-Yazied A (2007). Effect of fertigation frequency from subsurface drip irrigation on tomato yield grown on sandy soil. Aust. J. Basic Appl. Sci. 1(3): 279-285.

Balliu A, Bani A, Karajani M, Sulçe S (2007). Environmental impacts of nitrogen concentration of tomato and pepper seedling's nutrient solution. Acta Hortic. 747: 495-502.

Basoccu L, Nicola S (1995). Supplementary light and pretransplant nitrogen effects on tomato seedling growth and yield. Acta Hortic. 396: 313-320.

Blanco FA (2001). Métodos apropiados de análisis estadísticos subsiguientes al análisis de varianza (ANDEVA). Agronomía Costarricense, 25: 53-59.

Bremner JM (1986). Total nitrogen in Methods of soil analysis. Madison, USA. Agronomy, 2: p. 9.

Cartelat A, Cerovic ZG, Goulas Y, Meyer S, Lelarge C, Prioul JL, Barbottin A, Jeuffroy MH, Gate P, Agati G, Moya I (2005). Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). Field Crops Res. 91: 35-49.

Chang SX, Robison DJ (2003). Nondestructive and rapid estimation of hardwood foliar nitrogen satus using the SPAD-502 chlorophyll meter. Forest Ecol. Manage. 181(3): 331-338.

Finnan JM, Burke JI, Jones MB (1997). A note on a non-destructive method of chlorophyll determination in wheat (*Triticum aestivum* L). Iran. J. Agr. Food Res. 36: 85-89.

Gandrup ME, Garcia FO, Fabrizzi KP, Echeverria HE (2004). Evolución de un índice de verdor en hoja para evaluar el status nitrogenado en trigo. *RIA*. http://www.inta.gov.ar/ediciones/ria/33_3/07.pdf. 33(3): 105-121.

Jia L, Chen X, Zhang F, Buerkert A, Romheld V (2004). Use of digital camera to assess nitrogen status of winter wheat in the Northen China plain. J. Plant Nutr. 27(3): 441-450.

Kawashima S, Nakatani M (1998). An algorithm for estimating chlorophyll content in leaves using a video camera. Ann. Bot. 81: 49-54.

Kieffer DL (2009). Chlorophyll meter SPAD 502. User's manual. SPECTRUM TECHNOLOGIES, ILLINOIS, USA. http://www.specmeters.com/pdf/2900.pdf.

Pagola M, Ortiz R, Irogoyen I, Bustince H, Barrenechea E, Aparicio-Trejo P, Lamsfus C, Lasa B (2009). New method to asses barley nitrogen nutrition status base don image colour analysis. Comparison with SPAD-502. Comp. Electron. Agric. 65: 213-218.

Pinkard EA, Patel V, Mohammed C (2006). Chlorophyll and nitrogen determination for plantation-grown *Eucalyptus nitens* and *E. globulus* using a non-destrictive meter. Forest Ecol. Manage. 223: 211-217.

Richardson AD, Duigan SP, Beryn GP (2002). An evaluation of noninvasive methods to estimate foliar chlorophyll content. New Phytologist, 153: 185-194.

Rostami M, Koochecki AR, Mahallati MN, Kafi M (2008). Evaluation of chlorophyll meter (SPAD) data for prediction of nitrogen status in corn (*Zea mays* L.). Am. Eurasian J. Agric. Environ. Sci. 3(1): 79-85.

Sainz RH, Echeverría HE (1998). Relación entre las lecturas del medidor de clorofila (Minolta SPAD 502) en distintos estadios del ciclo del cultivo de maíz y el rendimiento en grano. Rev. Fac. Agron. La Plata, 103(1): 37-44.

SAS Institute Inc (1990). SAS/STAT User's Guide, Version 6, 4th ed. SAS Institute, Cary, NC.

Sexton P, Carrol J (2002). Comparison of SPAD chlorophyll meter readings vs. petiole nitrate concentration in sugarbeet. J. Plant Nutr. 25(9): 1975-1986.

Snedecor WG, Cochran GW (1976). Statistical Methods Applied to Experiments in Agriculture and Biology, 5th ed. Iowa State University Press, Ames, Iowa.

Spiegel MR (1992). Correlation Theory. Ch. 14 in Theory and Problems of Probability and Statistics, 2nd ed. New York: McGraw-Hill.

Steiner AA (1984). The universal nutrient solution. In proceeding of the Sixth International Congress on Soilless Culture. International Society for Soilless Culture. Lunteren. The Netherlands.

Wang Q, Chen J, Li Y (2004). Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. J. Plant Nutr. 27: 557-569.

Wiwart M, Fordonski G, Zuk-Golaszewska K, Suchowisska E (2009). Early diagnostics of macronutrient deficiencies in tree legume species by color image analysis. Comput. Electron. Agric. 65: 125-132.