Critical values of nitrogen indices in tomato plants grown in soil and nutrient solution determined by different statistical procedures[1]

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Abstract – The objective of this study was to establish critical values of the N indices, namely soil-plant analysis development (SPAD), petiole sap $N\text{-}NO_3$ and organic N in the tomato leaf adjacent to the first cluster (LAC), under soil and nutrient solution conditions, determined by different statistical approaches. Two experiments were conducted in randomized complete block design with four replications. Tomato plants were grown in soil, in 3 L pot, with five N rates (0, 100, 200, 400 and 800 mg kg^{-1}) and in solution at N rates of 0, 4, 8, 12 and 16 mmol L^{-1}. Experiments in nutrient solution and soil were finished at thirty seven and forty two days after transplanting, respectively. At those times, SPAD index and petiole sap $N\text{-}NO_3$ were evaluated in the LAC. Then, plants were harvested, separated in leaves and stem, dried at 70ºC, ground and weighted. The organic N was determined in LAC dry matter. Three statistical procedures were used to calculate critical N values. There were accentuated discrepancies for critical values of N indices obtained with plants grown in soil and nutrient solution as well as for different statistical procedures. Critical values of nitrogen indices at all situations are presented.

Index terms: *Lycopersicon esculentum*, nitrogen fertilizers, plant soil relations, statistical analysis.

Níveis críticos de índices de nitrogênio no tomateiro em solo e solução nutritiva determinados por diferentes procedimentos estatísticos

Resumo – O objetivo deste trabalho foi estabelecer os níveis críticos dos índices de N, SPAD ("soil-plant analysis development"), $N\text{-}NO_3$ na seiva do pecíolo e N orgânico na folha adjacente ao primeiro cacho do tomateiro (FAC), em solo e em solução nutritiva, determinados por diferentes procedimentos estatísticos. Foram conduzidos dois experimentos no delineamento em blocos casualizados, com quatro repetições. As plantas foram cultivadas em vasos contendo 3 L de solo, com cinco níveis de N (0, 100, 200, 400 e 800 mg kg^{-1}) e, também, em solução nutritiva com 0, 4, 8, 12 e 16 mmol L^{-1} de nitrogênio. Os experimentos em solução e em solo foram encerrados aos 37 e 42 dias após o transplante, respectivamente. Nesta ocasião, o índice SPAD e o teor de $N\text{-}NO_3$ foram determinados na FAC. Em seguida, as plantas foram colhidas, separadas em folhas e pecíolos, secadas a 70ºC, moídas e pesadas. Na matéria seca da FAC, foi determinado o N orgânico. Três procedimentos estatísticos foram utilizados para calcular o nível crítico. Verificaram-se acentuadas discrepâncias no valor do nível crítico dos índices de N obtidos com as plantas em solo e em solução nutritiva assim como utilizando-se diferentes procedimentos estatísticos. São apresentados os níveis críticos dos índices de N nas diversas situações.

Termos para indexação: *Lycopersicon esculentum*, adubo nitrogenado, relação planta-solo, análise estatística.

Introduction

Tomato (*Lycopersicon esculentum* Mill) is an important Brazilian vegetable crop having the nitrogen (N) a major influence on the productivity levels. The goal of N management program should be supplying enough N to achieve maximum profit from the crop. But any N not used by the tomato crop is potentially subjected to leaching, which pollutes groundwater and decreases the efficiency of N fertilization. Efficient N management program in tomato production can be attained by suitable evaluation of plant nutritional status (Coltman, 1988; Smith & Loneragan, 1997), usually accomplished by

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a quantitative analysis for the total N concentration, involving dry ashing procedure.

Alternatively to the total N determination, quick procedures have been proposed to evaluate tomato N status as the petiole-sap nitrate (Coltman, 1988; Hochmuth, 1994; Guimarães et al., 1998) and the leaf greenness determination by a hand-held device called Minolta SPAD (soil-plant analysis development) meter (Guimarães et al., 1999; Sandoval-Villa et al., 1999). SPAD index measured on plant leaves were positively correlated to N sufficiency for several crops (Piekielek & Fox, 1992; Blackmer & Scherpers, 1995; Shapiro, 1999; Rodrigues et al., 2000) and can be accepted as an N index.

Independently of the N index, the existence of values accepted as the critical N concentration is necessary to be used as a standard or reference. Usually, recommendation for critical values to evaluate N status are derived from tomato field survey and or field and greenhouse studies at soil and nutrient solution conditions in which tomato plant responses to a range of fertilizer rates are measured. Decision concerning optimal values involves fitting by a model that describes well the data. Model selection is a major factor affecting which rate is identified as being optimal (Cerrato & Blackmer, 1990).

A limited survey of published reports indicates several models for best describing crop response to N fertilization, which depends on the N rates high enough to produce a clear maximum response, spaced N levels to adequately model the level-response relationship, appropriated plant growth, N absorption rates and fruit yield and utilization of variance analysis to estimate nutrient requirement from level response data. As a result, numerous standard N index values are published. Besides the chosen model as found for N rates (Cerrato & Blackmer, 1990), type and age of tissue sampled (Mills & Jones Junior, 1996; Smith & Loneragan, 1997), and soil type (Guimarães et al., 1999), selected yield level and substrate characteristics may affect the values to be used as a reference or critical level.

The objectives of this study were to establish critical ranges of N indices, namely SPAD, petiole sap $N\text{-}NO_3$, and organic N, in the tomato leaf adjacent to the first cluster under soil and nutrient solution conditions, estimated by different statistical approaches.

Material and Methods

Two experiments were carried out at greenhouse conditions in the Universidade Federal de Viçosa, MG, Brazil. The first was in soil, and the second in nutrient solution with tomato cultivar Santa Clara, in a randomized complete-block design with four replicates.

The first experiment was conducted on samples of the surface 20 cm of a silt clay soil. Soil samples were sieved thorough a 1 x 1 cm opening screen, air dried, limed with 2 g dm^{-3} and fertilized with P (435 mg kg^{-1}) and K (100 mg kg^{-1}). Five N levels were established, 0, 100, 200, 400 and 800 mg kg^{-1} by mixing $(NH_4)_2SO_4$ with the soil. Then, the soil was placed into 3 kg pots. One tomato seedling was transplanted into each pot.

The aerated nutrient solution experiment was conducted in 8 L pots containing P, K, Ca, Mg and S at 2, 5.5, 4.5, 2 and 4 mmol L^{-1}, respectively, and micronutrient concentrations following Hoagland & Arnon (1950). Five N rates, 0, 4, 8, 12, and 16 mmol L^{-1} were evaluated, being 20% as $N\text{-}NH_4$ and 80% as $N\text{-}NO_3$. The solution was adjusted daily to pH 5.8±0.3 with NaOH or HCl and the pots volumes were completed with deionized water. One tomato seedling was transferred to each pot.

After the beginning of the tomato plant reproductive phase, 37 and 42 days after seedling transplanting to solution and soil experiments, respectively, petiole-sap nitrate (PSN) and SPAD (soil-plant analysis development) indices were measured in the leaf adjacent to the cluster (LAC). A Minolta SPAD 502 meter was used to take chlorophyll readings, taken on the terminal leaflet of the LAC. Then, the LAC petiole base was cut at 2 cm from its insertion in the stem and crushed in a stainless steel garlic crusher. Sap was collected on the meter's electrode (C-141 Cardy Nitrate Meter - HORIBA, Inc.) and the $N\text{-}NO_3$ concentration was read at the digital meter. Then, the LAC was harvested, dried, ground to pass a 1 mm sieve, ashed with sulphuric acid and analyzed colorimetrically for organic N (Jackson, 1958). After SPAD and PSN determinations, the plant top was cut off at the cotyledonary node, and dried in a forced air oven at 70°C, and the shoot dry weight (SDW) was recorded.

In each experiment, N effect levels on PSN, SPAD index, N concentration in LAC dry matter (ORN) and SDW were analyzed by analysis of variance. Next, three statistical procedures were used to calculate the critical N indices. By the procedure named one, linear, quadratic, square root, potential, exponential, hyperbolic, logarithmic and cubic root models were fitted to statistically significant data using N level as the independent variable. The best fitting model with biological logic was used to estimate the maximum SDW obtained by equating the first derivatives of the best fitting model to zero, solving for X, substituting the X values into the model and solving for Y. To estimate PSN, SPAD index and ORN critical values in both experiments, N rate associated to maximum SDW (CV_{100}) was introduced into the best fit model previously determined, which correlates PSN, SPAD index and ORN to N level. The model also was used to determine the PSN, SPAD index and ORN critical values associated to 99.9, 99, 95, and 90% of the maximum SDW.

By the procedure named two, the steps were the same as in number one, but the best fitting model, with biological logic, was chosen among only linear, quadratic and cubic models.

By the procedure named three, all models listed in the procedure number one were fitted to PSN, SPAD index and ORN as independent variables (X) and the SDW as the dependent variable (Y). In each experiment, the best fitting model with biological logic within the range of observed X values was used to estimate PSN, SPAD index and NDM critical values at CV_{100}, $CV_{99.9}$, CV_{99}, CV_{95}, and CV_{90}.

Results and Discussion

By the procedure named one, tomato shoot dry weight (SDW) responded ($p<0.01$) to applied N levels until they reached maximum values of 21.67 and 27.75 g plant^{-1} at 172 mg kg^{-1} and 6.35 mmol L^{-1} in soil and nutrient solution, respectively (Figure 1). By the procedure named two, the corresponded values were 21.71 and 26.97 g plant^{-1} at 351 mg kg^{-1} and 9.43 mmol L^{-1}, respectively (Figure 1). As expected, less than maximum shoot dry weight was obtained with lower N rates (Table 1). Several authors found negative effects of high N levels in soil (Guimarães et al., 1999) and in nutrient solution (Fontes et al., 1995) on tomato SDW. N nutrition enhances metabolic processes that influence the physicochemical environment at the soil-root interface, interfere with the uptake of cations and anions, enhance or repress several enzyme system activities, and affect plant growth patterns (Fernandes & Rossielo, 1995). High $N\text{-}NO_3$ levels decrease important aminoacids formation and change the vacuolar pH due to $N\text{-}NO_3$ accumulation (Mohamed et al., 1987) and high $N\text{-}NH_4$ levels disrupt biological membranes, uncouple photophosphorylation, block ATP production, reduce CO_2 fixation and decrease nutrient absorption, mainly Ca, Mg, and K (Claassen & Wilcox, 1974).

The two models predicted similar maximum SDWs but N levels to achieve them in soil and nutrient solution were in average 104% and 48% higher when procedure two was utilized instead of procedure one (Table 1). Tomato plants grown in solution were more efficient to utilize N than plants in soil. In solution,

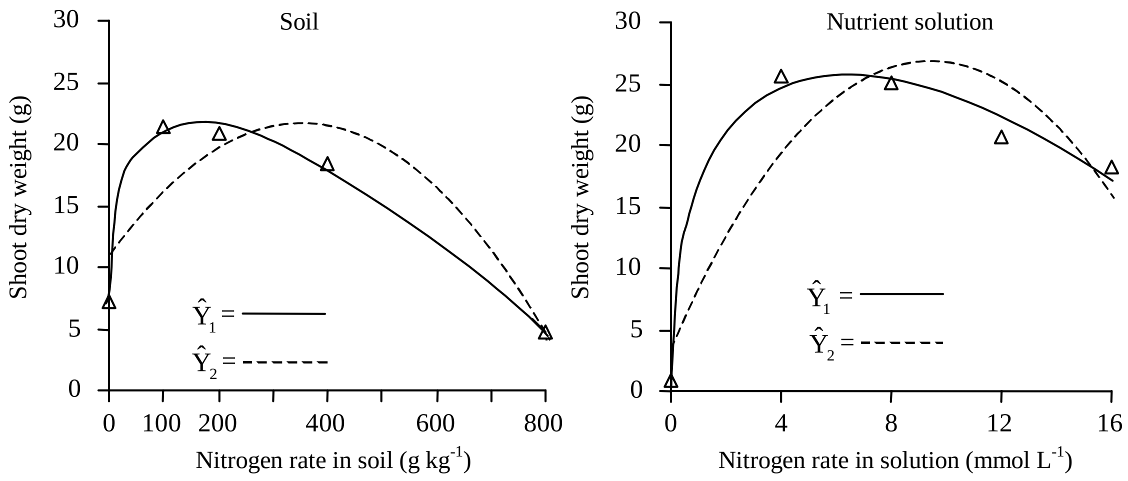

Figure 1. Relationships between tomato plant shoot dry weight and nitrogen application rate in soil established by procedures named one ($\hat{Y}_1 = 7.263 + 2.259X^{0.5} - 0.0933X + 0.000366X^{1.5}$, $R^2 = 0.996$) and two ($\hat{Y}_2 = 10.998 + 0.0610X - 0.0000871X^2$, $R^2 = 0.794$) and in nutrient solution established by procedures one ($\hat{Y}_1 = 0.993 + 19.635X^{0.5} - 3.893X$, $R^2 = 0.989$) and two ($\hat{Y}_2 = 3.762 + 4.9216X - 0.2605X^2$, $R^2 = 0.811$).

estimated by model one, the maximum tomato SDWs per g of added N was 312 mg and only 126 mg in soil, that is 148% higher. The corresponded value estimated by model two was 229% higher. Depending on the growth conditions, N efficiency of young tomato plant would range from 27 (Sampaio et al., 1995) to 332 mg g^{-1} (Guimarães et al., 1999).

Estimated by procedures one and two, petiolesap nitrate levels in tomato plants grown in soil (Figure 2) and SPAD indices (Figure 3) and organic-nitrogen (Figure 4) values in plant grown in both soil and nutrient solution were increased ($p<0.01$) by increasing N rate up to determined value within the experimental space; petiole-sap nitrate levels in plants under nutrient solution conditions were increased ($p<0.01$) by N rates. Sap test measures $N\text{-}NO_3$ present in xylem and phloem sap plus the apoplastic, citosolic and vacuolar water. It is a direct

Table 1. Tomato shoot dry weight (SDW) and nitrogen levels (NL) in soil and in nutrient solution predicted by procedures named one and two, at several percentage of the maximum.

Variable	Procedure number[(1)]	Percent of the maximum 100	99.9	99	95	90
		Soil (g $plant^{-1}$)				
SDW	One	21.67	21.65	21.45	20.58	19.50
	Two	21.71	21.69	21.50	20.63	19.54
		Nutrient solution (g $plant^{-1}$)				
	One	25.75	25.73	25.50	24.47	23.18
	Two	26.97	26.94	26.70	25.62	24.27
		Soil (mg kg^{-1})				
NL	One	172	158	132	89	63
	Two	351	334	301	203	193
		Nutrient solution (mmol L^{-1})				
	One	6.36	5.96	5.13	3.79	2.92
	Two	9.43	9.11	8.41	7.15	6.21

[(1)]In the procedure number one the best fitting model selected among linear, quadratic, square root, potential, exponential, hyperbolic, logarithmic and cubic root models was used; in the procedure number two only linear, quadratic and cubic models were used.

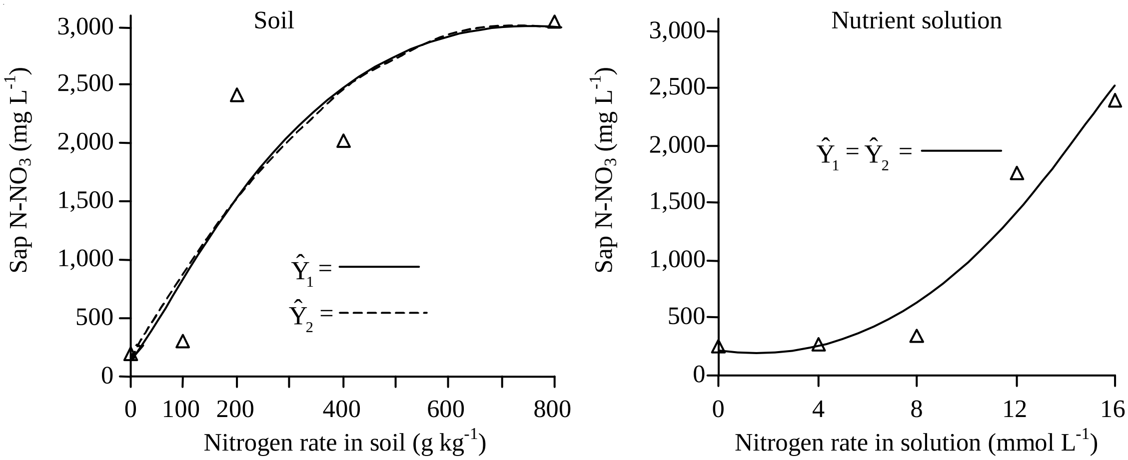

Figure 2. Relationships between sap $N\text{-}NO_3$ in tomato leaf adjacent to the first cluster and nitrogen application rate in soil established by procedures named one ($\hat{Y}_1 = 146.425 - 40.498X^{0.5} + 14.711X - 0.344X^{1.5}$, $R^2 = 0.803$) and two ($\hat{Y}_2 = 153.615 + 7.920X - 0.00547X^2$, $R^2 = 0.796$) and in nutrient solution by procedures one ($\hat{Y}_1$) and = two ($\hat{Y}_2$) = $221.357 - 40.991X + 11.574X^2$, $R^2 = 0.943$).

measure of current N supply and is markedly affected by many factors among them the light intensity (Fukuda et al., 1999).

The SPAD index of plant leaves supplied with high amount of N were significantly greater than when low amount or no N was applied (Figure 3). It was also greater in plants grown in soil than in solution. The SPAD index detects the transmittance of light emitted by two diodes, one with a peak absorbance at 650 nm and the other one at 940 nm. In the first one, there was high light absorptance by chlorophyll and in the second one light absorptance was

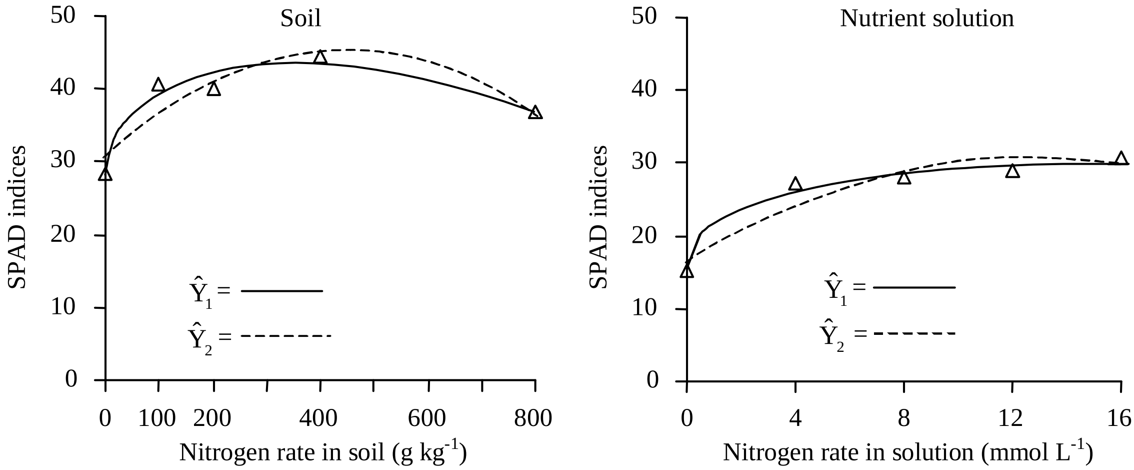

Figure 3. Relationships between SPAD (soil-plant analysis development) indices in tomato leaf adjacent to the first cluster and nitrogen application rate in soil established by procedures named one ($\hat{Y}_1 = 28.409 + 1.246X^{0.5} - 0.00549X - 0.000985X^{1.5}$, $R^2 = 0.945$) and two ($\hat{Y}_2 = 30.639 + 0.0665X - 0.0000739X^2$, $R^2 = 0.827$) and in nutrient solution by procedures one ($\hat{Y}_1 = 15.371 + 7.308X^{0.5} - 0.917X$, $R^2 = 0.986$) and two ($\hat{Y}_2 = 16.551 + 2.289X - 0.0923X^2$, $R^2 = 0.898$).

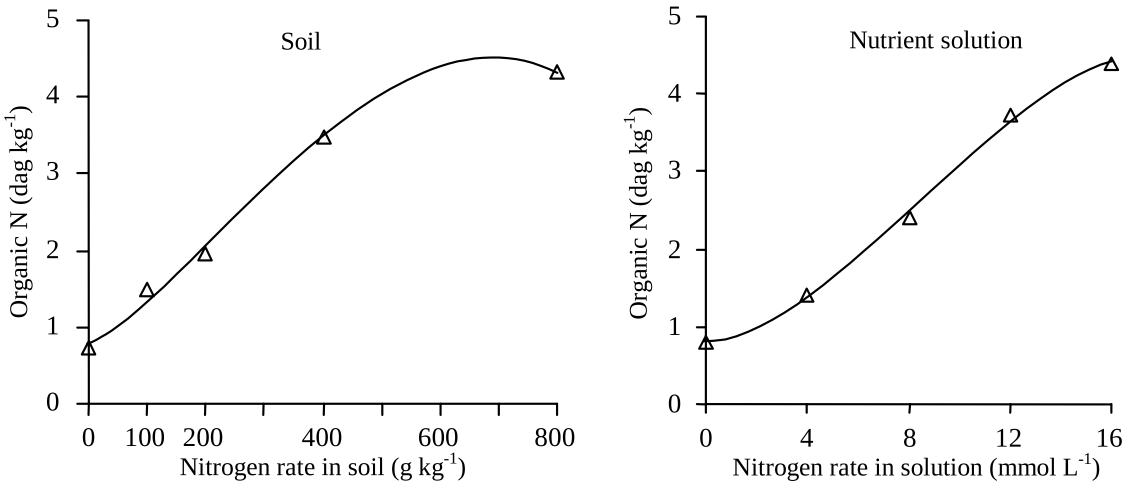

Figure 4. Relationships between organic nitrogen in tomato leaf adjacent to the first cluster and nitrogen application rate in soil established by procedures named one ($\hat{Y}_1$) and = two ($\hat{Y}_2$) = $0.781 + 0.00496X + 0.00000945X^2 - 0.00000000127X^3$, $R^2 = 0.997$) and in nutrient solution by procedures one ($\hat{Y}_1$) and = two ($\hat{Y}_2$) = $0.807 + 0.0244X + 0.0335X^2 - 0.00131X^3$, $R^2 = 0.998$).

negligible. So, the SPAD index represents the light transmittance ratio through the leaf tissue at those wavelengths and may be used to provide a rapid estimate of leaf transmittance and reflectance in the field (Madeira et al., 2000).

Peculiar to plant species (Marquard & Tipton, 1987) and growth conditions (Campbell et al., 1990; Guimarães, 1998), positive relationship has been demonstrated between SPAD readings and chlorophyll contents in leaves. With increase in chlorophyll content, light absorption by plant leaves increases. Chlorophyll is responsible for leaf greenness and generally recognized as an indication of N status for many crops. Intensity of green colour leaves has been used as an index of N concentration in the leaves (Takebe et al., 1990) as long as the concentration of N in nitrate form be low. Ali et al. (1999) found that N-deficient yellowish leaves contained small amounts of RuBisCO but when excess of N fertilizer is applied, the leaves function was significantly less efficient in spite of high chlorophyll and RuBisCO contents.

Tomato leaf green color was less intense in solution culture than in soil as indicated by the SPAD values (Figure 2). Due to position in the greenhouse, plants grown in nutrient solution were more shaded than in soil influencing the light irradiance on tomato which may affect chloroplast orientation in leaves (Hoel & Solhaug, 1998). Furthermore, in solution P, Mg, Mn, Zn, Cu, Fe, S, and K concentrations in leaves were lower than in leaves of tomato plants grown in soil. The inverse was true for Ca and B. Partial Ca and Mg deficiencies but K decreased chlorophyll contents in lemon leaves (Lavon et al., 1999).

Relationships between N indices and tomato shoot dry weight (SDW), under soil and nutrient solution, which were utilized for critical level determination by procedure named three, are presented (Table 2). In all situations, but SPAD indices in plants grown in soil, SDW values increased with increasing N indices up to a maximum point which was the critical value (CV_{100}) estimated by procedure named three (Table 3). CV_{100} for SPAD index in soil was 44.4, estimated with the maximum observed SDW value (22.15 g plant^{-1}).

As expected, all critical N indices in tomato plants grown in soil and nutrient solution were higher when 100% maximum shoot dry weight was selected (Table 3). Higher maximum values selection implied in relatively low decrease in shoot dry weight and high decrease in N rates (Table 1). There were considerable disagreement among the statistical procedures, substrates and yield levels selected to estimate critical N indices in tomato leaf, indicating a need to emphasize them when setting critical values (Table 3).

As the price of N fertilizer decreases relatively to the price of tomato fruit, high percentage of the maximum yield should be chose but fertilization levels of N to give 100% of fruit yield usually are not economic and/or ecological optimal. In this paper, it is assumed that N index estimated by procedures one and three for 99% of the maximum shoot dry weight are the critical range values (CV_{99}).

Table 2. Nitrogen indices (X) in tomato leaf adjacent to the first cluster related to shoot dry weight (g plant^{-1}) in soil and nutrient solution.

N indices (X)	Regression equations	R^2
	Soil	
Sap N-NO_3 (mg L^{-1})	$\hat{Y} = 9.564 + 0.0266X - 0.0000120X^2$	0.558
SPAD[(1)] index	$\hat{Y} = 206.530 - 77.0726X^{0.5} + 7.414X$	0.545
N in leaf DM[(2)] (dag kg^{-1})	$\hat{Y} = -17.025 + 1.5409X^{0.5} + 50.287X - 22.0837X^{1.5}$	0.959
	Nutrient solution	
Sap N-NO_3 (mg L^{-1})	$\hat{Y} = -20.727 + 2.664X^{0.5} - 0.0359X$	0.427
SPAD index	$\hat{Y} = -611.473 + 260.1946X^{0.5} - 26.469X$	0.989
N in leaf DM (dag kg^{-1})	$\hat{Y} = -102.045 + 163.2808X^{0.5} - 50.827X$	0.876

[(1)]SPAD: soil-plant analysis development. [(2)]DM: dry matter.

In soil, PSN critical concentrations for CV_{99} estimated by procedures one and three ranged from 966 to 1,098 mg L^{-1} (Table 3). Corresponding values in nutrient solutions were 315 to 1,175 mg L^{-1} (Table 3). Published values of optimum PSN are 1,000 to 1,200 (Hochmuth, 1994); 1,091 (Coltman, 1987); 960 to 1,160 in spring tomato crop or 776 to 996 for the fall crop (Rhoads et al., 1996); and 2,581 mg L^{-1} in situation with six N applications (Guimarães et al., 1998).

Critical SPAD indices for CV_{99} estimated by procedures one and three ranged from 40.3 to 44.2 in plants under soil conditions and the corresponding values in plants grown in nutrient solution were 27.2 to 23.2 (Table 3). SPAD index measures leaf greenness ranging from 0 to 80 with a higher number representing a greener leaf (Dwyer et al., 1995). At two different soil type, SPAD critical values in tomato leaf adjacent to the cluster measured at flowering stage were 35.5 and 46.5 (Guimarães et al., 1999). SPAD values of 43.4 and 52.0 in corn were established to distinguish between responsive and non-responsive site to sidedress N (Piekielek & Fox, 1992; Smeal & Zhang, 1994; Piekielek et al., 1995).

In plants grown in soil, leaf organic N critical concentrations for CV_{99} estimated by procedures one and three ranged from 1.57 to 2.07 dag kg^{-1} and the corresponding values in plants grown in nutrient solution were 1.63 to 2.34 dag kg^{-1} (Table 3). These values are lower than 2.80 to 4.20 dag kg^{-1} indicated by Mills & Jones Junior (1996) for tomato plants under greenhouse conditions. There are also lower than 3.02 and 3.43 dag kg^{-1} found by Sampaio et al.

Table 3. Critical values of nitrogen indices in tomato leaf adjacent to the first cluster (LAC) associated with different percentages of the maximum shoot dry weight in soil and nutrient solution determined by three different statistical procedures.

N index	Procedure number[(1)]	Percent of the maximum 100	99.9	99	95	90
				Soil		
Sap $N\text{-}NO_3$ (mg L^{-1})	One	1,370	1,283	1,098	786	580
	Two	2,260	2,193	2,042	1,735	1,478
	Three	1,108	1,064	966	789	658
				Nutrient solution		
	One	428	390	315	232	200
	Two	864	808	695	521	413
	Three	1,377	1,313	1,175	947	792
				Soil		
SPAD[(2)] index	One	41.6	41.3	40.3	38.9	37.5
	Two	44.9	44.6	44.0	42.3	40.7
	Three	44.4	44.3	44.2	43.7	42.9
				Nutrient solution		
	One	28.0	27.8	27.2	26.1	25.2
	Two	29.9	29.7	29.3	28.2	27.2
	Three	24.2	23.8	23.2	21.9	21.2
				Soil		
N in leaf dry matter (dag kg^{-1})	One	1.85	1.75	1.57	1.29	1.13
	Two	3.14	3.03	2.78	2.33	2.00
	Three	2.27	2.21	2.07	1.83	1.65
				Nutrient solution		
	One	1.98	1.87	1.63	1.31	1.13
	Two	2.92	2.82	2.60	2.22	1.94
	Three	2.58	2.50	2.34	2.06	1.87

[(1)]In the procedure number one the best fitting model selected among linear, quadratic, square root, potential, exponential, hyperbolic, logarithmic and cubic root models was used; in the procedure number two, only linear, quadratic and cubic models were used; in the procedure number three the same models were used as in the procedure number one, being all this nitrogen indices as independent variable (X), and the shoot dry weight as the dependent variable (Y). [(2)]SPAD: soil-plant analysis development.

(1995) and Guimarães et al. (1998) for tomato plants grown in nutrient solution and soil under greenhouse conditions, respectively. Several reasons led to different critical levels being the timing of N application, that is N availability, and the sink demand or dry matter yield the most significatives. In both experiments, tomato plants were harvested before fruit setting and it would be interesting to test if so small N concentrations in leaf tissue maintained through the entire plant cycle by daily or weekly N additions would led to profitable fruit yield.

Conclusion

Critical values of nitrogen indices in tomato plant leaf depend on substrate and statistical procedure utilized.

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