Rapid NPK diagnosis in tomato using petiole sap analysis with the DRIS method

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ABSTRACT

One of the factors determining success in tomato production is plant nutrition. The diagnosis and recommendation integrated system (DRIS) is a tool used to obtain fertilizer recommendations. The objective of this study was to determine DRIS indices using petiole sap analysis (PSA) and compare them with those obtained using leaf analysis in two tomato cultivars grown under greenhouse conditions. A complete randomized design with eight treatments and 60 plants per experimental unit was used. To determine whether employing PSA with the DRIS methodology can provide a reliable diagnosis of fertilization, doses of some elements, such as N, P, or K, were reduced by 30% and supplied to two tomato cultivars, namely, Cid and Moctezuma. The indices for PSA-DRIS and DRIS were estimated. R^2 values greater than 0.84 were obtained for the relationships between the leaf concentrations and petiole extract (PE) concentrations of some elements in both tomato cultivars. The PSA-DRIS method detected 62.5% of the cases of deficiency identified via DRIS. The PSA-DRIS method is a low-cost way to rapidly fertilize recommendations in tomato.

Keywords: *Solanum lycopersicum*, petiole extract, nutritional balance, nutrients.

RESUMO

Diagnóstico rápido de NPK em tomate por meio da análise da seiva de pecíolos e pelo método DRIS

Um dos fatores que determina o sucesso na produção de tomate é a nutrição das plantas. O sistema integrado de diagnóstico e recomendação (DRIS) é uma ferramenta usada para obter recomendações de fertilizantes. O objetivo deste estudo foi determinar os índices DRIS usando análise de seiva de pecíolo (PSA) e compará-los com os obtidos pela análise foliar em duas cultivares de tomate cultivadas em casa de vegetação. O delineamento experimental foi inteiramente casualizado, com oito tratamentos e 60 plantas por unidade experimental. Para determinar se o emprego de PSA com a metodologia DRIS pode fornecer um diagnóstico confiável de fertilização, as doses de alguns elementos, como N, P ou K, foram reduzidas em 30% e fornecidas a duas cultivares de tomate, Cid e Moctezuma. Os índices para PSA-DRIS e DRIS foram estimados. Valores de R^2 superiores a 0,84 foram obtidos para as relações entre as concentrações foliares e extrato de pecíolo (PE) de alguns elementos em ambas as cultivares de tomate. O método PSA-DRIS detectou 62,5% dos casos de deficiência identificados via DRIS. O método PSA-DRIS é uma maneira de baixo custo para recomendação rápida de fertilização do tomate.

Palavras-chave: *Solanum lycopersicum*, extrato do pecíolo, balanço nutricional, nutrientes.

The efficient use of fertilizers in tomato crops is important not only to reduce production costs but also to minimize possible negative effects on the environment (Hochmuth *et al.*, 2004). Foliar analysis is considered very practical for the diagnosis of nutritional disorders (Dezordi *et al.*, 2016) and is superior to soil analysis for determining the nutritional status of a crop as soil analysis only determines the nutrients immediately available in the soil (Shunfeng *et al.*, 2018). However, foliar analysis has the disadvantages of being expensive and requiring several days to conduct. In addition, the levels of leaf nutrients are influenced by concentration effects due to limiting factors in development and dilution effects due to culture medium conditions (Cadahía, 2008).

As an alternative, sap analysis, also known as petiole sap analysis (PSA), is a quick and effective tool for determining the nutritional status of a crop. This method is based on the measurement of the concentrations of nutrients in the leaf petiole sap (Bityutski *et al.*, 2017). In PSA, before sap extraction, the petioles should be sampled in the field, managed and stored following standard protocols (Araya *et al.*, 2015), then analyzed immediately to avoid changes in nutrient contents (Thompson *et al.*, 2009). According to Cadahía (2008), sap concentrations are less affected by concentration and dilution effects than are foliar concentrations; thus, sap analysis offers a diagnosis that not only is more accurate than that offered by foliar analysis but also requires less time. Several types of

equipment are available for measuring the concentrations of ions in plant sap, such as colorimeters and specific ion electrodes (Hochmuth *et al.*, 2004), that yield values with strong correlations (r^2=0.96, p<0.0001) with those obtained from conventional laboratory analyses (Tully & Weil, 2014). This equipment has been used successfully in various crops, such as tomato (Hochmuth *et al.*, 2004; Farneselli *et al.*, 2014) and leafy vegetables such as lettuce, spinach, and Asian vegetables (Ott-Borrelli *et al.*, 2009; Parks *et al.*, 2012). However, it is difficult to interpret the results of sap analysis of crops, in particular, it is difficult to determine whether a nutrient is present in excess or is deficient and thus negatively affecting yield.

One of the best methods for the interpretation of foliar analysis results is the diagnosis and recommendation integrated system (DRIS) (Beaufils, 1973; Walworth & Sumner, 1987). The DRIS evaluates the nutritional status of a plant by comparing the relative proportions of nutrient pairs in the foliar tissue with published standards for the crop (Soltanpour *et al.*, 1995). The results are expressed as indices representing, on a numerical scale, the effects of individual nutrients on the nutritional balance of the plant (Bhaduri & Pal, 2013).

Considering that DRIS uses the concept of nutritional balance and is suitable for the detection of deficiencies and/or nutritional excesses (Bhaduri & Pal, 2013) and that sap analysis is a very useful and rapid method for the detection of xylem and phloem concentrations of nutrients in a cultivar (Farneselli *et al.*, 2014), the objective of this study was to compare the DRIS indices obtained based on foliar analysis with those obtained based on PSA in two tomato cultivars.

MATERIAL AND METHODS

The experiment was conducted from March to December 2018 at the Universidad Autónoma de Querétaro, Mexico (20°11'17"N, 100°08'38"W, altitude 2629 m). The tomato (*Solanum lycopersicum*) cultivars used were Cid and Moctezuma from the Harris Moran Company (USA) and were grown under greenhouse conditions.

The tomato plants were grown hydroponically in blocks of rock wool (1x0.2x0.075 m) at a density of 2.5 plants m^{-2} (0.4 m separation between each block of rock wool, 1.6 m between furrows). The nutrient solution used was Steiner solution (Steiner, 1984) with a pH of 5.8 containing the following in mg L^{-1}: N, 167; P, 31; K, 277; Mg, 49; Ca, 183; S, 111; Fe, 1.33; Mn, 0.62; B, 0.44; Cu, 0.02; Zn, 0.11; and Mo, 0.048. The nutrient solution was applied into two stages according to Ávila-Juárez *et al.* (2015). The temperature, relative humidity, and total solar radiation were 30.5/10.5°C (day/night), 60.4/33.7% (day/night), and 955 W m^{-2} (maximum), respectively, during the experiment.

To achieve known nutritional deficiencies for the treatments, N, P, or K was reduced by 30% in the nutrient solution. Two cultivars (Cid and Moctezuma) were used, yielding a total of eight treatments, including control treatments, in which nutrients were not reduced. Sixty plants comprised each experimental unit. The experimental design was completely randomized. The treatments were coded as T1 (-NCid), T2 (-PCid), T3 (-KCid), T4 (-NMoc), T5 (-PMoc), T6 (-KMoc), T7 (control treatment; controlCid for N, P, or K), and T8 (control treatment; controlMoc for N, P, or K), where T= treatment, N= nitrogen, P= phosphorus, K= potassium, Moc= Moctezuma cultivar, and Cid= Cid cultivar.

For the extraction of petiole sap, the fifth developed young leaf (n= 60) from the top of the plant was collected (9:00 am). Petiole extract (PE) sampling and foliar analyses were performed when the plant presented cluster number five (120 days after transplant). Subsequently, for the analysis of PE, the leaflets were removed from the leaf, leaving only the petioles, which were cut to 0.5 cm in length and frozen for 24 h. The PE was extracted with a hydraulic press and diluted to a ratio of one part PE to 90 parts deionized water. The dilution was prepared according to the equipment specifications and immediately analyzed by a colorimeter model HI83225 (Hanna Instruments, USA). N (NO_3-N) was determined by the cadmium reduction method, P (PO_4-P) was determined by the amino acid method, and K was determined by the tetraphenylborate turbidimetric method.

For foliar analysis, leaves (n= 60) were collected from different parts of the plant and then immediately analyzed in a certified laboratory (Laboratorio de Nutrición Vegetal SC) in Guanajuato, Mexico. N was determined according to the Kjeldahl method, and K was determined by atomic absorption followed by wet digestion. P was determined by spectrophotometry after digestion and calcination according to Ávila-Juárez *et al.* (2015).

To determine the deficiency induced by each treatment presented as a percentage, the result for each element (N, P or K) in leaf or PE was multiplied by 100 and then divided by the control value (without deficiency); then, 100 was subtracted. This procedure was performed to compare the laboratory results with the PSA results.

The DRIS standards for greenhouse tomato were provided by the Mexican National Institute of Forestry, Agriculture and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias - INIFAP) and were N/P= 8.947, coefficient of variation (CV)= 30; N/K= 1.063, CV= 20; and P/K= 0.119, CV= 25.

The DRIS and PSA-DRIS indices were calculated for each treatment in two steps. First, for each pair of nutrients, the observations were related to the existing norms by calculating the standardization index according to Beaufils (1973) as follows:

$$f(N/P) = \left(\frac{N/P}{n/p} - 1\right)\left(\frac{1000}{CV_{n/p}}\right)$$

$$if\ N/P > n/p \quad (1)$$

$$f(N/P) = \left(1 - \frac{n/p}{N/P}\right)\left(\frac{1000}{CV_{n/p}}\right)$$

$$if\ N/P < n/p \quad (2)$$

where the lowercase letters refer to the nutrient ratios of the published standards, uppercase letters refer to the samples of the foliar analysis or PSA, f is the function of the sample, and CV is the coefficient of variation associated with the standard. Once the

functions were calculated, we calculated the indices for each element as follows (Walworth & Summer 1987):

$$A = \frac{\pm f(A/B) \pm f(A/C) \pm f(A/D)...\pm f(A/N)}{z}$$

where f(A/B) is the ratio function of nutrients A and B. The symbol ± indicates the following: if A is the numerator in the function f(A/B), addition is performed; otherwise, subtraction is performed (Píperas *et al.*, 2009); the same approach applies to the remaining nutrient ratios. Z is the number of functions present in the A index.

RESULTS AND DISCUSSION

The laboratory analyses revealed a significant difference between each deficiency treatment and the corresponding control (without deficiency) except for N in the Moctezuma cultivar, confirming the induction of deficiency. To compare the laboratory and PSA approaches, the data were standardized using the percentage deficiency (as described in the material and methods section). Our results confirm that colorimetric analysis is capable of detecting nutrient deviations as traditional analysis can but more quickly (Table 1). In fact, there was no significant difference in the percentage of induced deficiency between the two methodologies. Colorimetry analysis of NO_3-N has been reported to be a reliable method for measuring N in PCE (Ott-Borrelli *et al.*, 2009), although it can vary with species and environmental conditions (Goffart *et al.*, 2008). Our results identified no significant differences in percentage deficiency for N or the other analyzed elements between the PSA method and the laboratory method. These results are significant because they show that equipment such as portable colorimeters can generate values equivalent to those obtained via laboratory analysis (with some uncertainty and high cost of $31 USD/sample), allowing a producer to rapidly (in less than 24 h) and economically (at less than $1.5 USD/sample) obtain information on the nutritional status of a crop.

P is especially important in the initial stages of crop development (Hopkins *et al.*, 2014); it is translocated from lower leaves to areas of new growth (Hochmuth *et al.*, 2004). Because P is a highly mobile element in plants, deficiencies (less than 0.1% dry weight) are visually perceptible in the oldest leaves (Hochmuth *et al.*, 2004). Values well above 0.1% weight based on dry matter were obtained in treatments where P deficiency was induced (T2 and T5), although they were significantly lower than the corresponding control values. The PSA method indicated up to 55% deficiency of P relative to the control percentage in the Cid cultivar, and this percentage was statistically equivalent to the percentage P deficiency indicated by the laboratory method. This result suggests that the P content in tomato plants can very feasibly be quantified via PSA.

The laboratory results revealed a significant difference between the K-deficiency treatment and the control treatment in both cultivars. However, there was no significant difference in the percentage of induced deficiency between the PCE method and the laboratory method. In the Cid cultivar, the laboratory analysis indicated an induced deficiency of 35.18%, whereas the PSA indicated a deficiency of 11.93%. The small amount of K in the petiole of the tomato leaf might reflect translocation that was too slow or

Table 1. Induced deficiencies and data variation between laboratory analysis (leaf analysis) and petiole sap analysis (PSA) in two tomato cultivars. Querétaro, UAQ, 2018.

Treatment	Laboratory		PSA	
	Lab (%)[w]	Deficiency[g] (%)	PE (ppm)[w]	Deficiency[g] (%)
-NCid	3.57NS	11.7±7.8[a]	816	39.9±19.8[a]
-NMoc	3.25**	20.0±4.8[a]	600	33.6±8.8[a]
-PCid	0.30**	53.7±13.5[a]	343	55.5±4.9[a]
-PMoc	0.38*	53.9±28.0[a]	426	43.3±28.6[a]
-KCid	2.76*	35.1±18.1[a]	1616	11.9±8.0[a]
-KMoc	3.18*	20.4±12.1[a]	1700	8.8±5.4[a]
control Cid N	4.05		1356	
control Moc N	4.06		910	
control Cid P	0.66		776	
control Moc P	0.83		783	
control Cid K	4.25		1840	
control Moc K	4.00		1866	

[g]Deficiency induced relative to the control level (mean ± standard deviation). [w]Comparison of induced deficiency in laboratory analysis vs. the respective control (e.g., -NCid vs. control Cid N); Tukey, p<0.05 (*) and p<0.01 (**). Moc= cv. Moctezuma; Cid= cv. Cid; -N= nitrogen deficiency, -P= phosphorus deficiency, -K= potassium deficiency. Means within a row with different letters are significantly different, Tukey, p<0.05.

Table 2. Relationships between the concentrations of nutrients in tomato leaf and those in petiole extract (PE) in two tomato cultivars (in treatments without induced deficiency). Querétaro, UAQ, 2018.

Cultivar	Relationship between foliar and PE concentrations			
	Element	Slope	Intercept	R²
Cid	A	4×10^{-4}	3.55	0.98
	P	-1×10^{-4}	0.77	0.97
	K	-5×10^{-7}	4.34	0.98
Moctezuma	N	6×10^{-4}	3.54	0.99
	P	2×10^{-4}	0.67	0.99
	K	-5×10^{-9}	4.15	0.84

Table 3. Indexes, excesses, and deficiencies as evidenced by DRIS and PSA-DRIS in two tomato cultivars. Querétaro, UAQ, 2018.

Treatment	DRIS			PSA-DRIS			Deficiency		Excess	
	I_N	I_P	I_K	I_N	I_P	I_K	DRIS	PSA-DRIS	DRIS	PSA-DRIS
-NCid	-19.2	10.7	8.5	-217.5	232.2	-14.7	N	N	P	P
-NMoc	-14.0	7.7	6.2	-222.9	204.9	18.0	N	N	P	P
-PCid	2.4	-16.1	13.7	-4.7	49.1	-44.4	P	K	K	P
-PMoc	-2.5	-7.0	9.5	-23.9	35.8	-11.9	P	N	K	P
-KCid	3.1	19.6	-22.7	-5.8	90.2	-84.5	K	K	P	P
-KMoc	-4.5	29.8	-25.3	-106.8	159.1	-52.3	K	N	P	P
control Cid	-10.5	13.6	-3.1	-79.7	119.6	-39.9	N	N	P	P
control Moc	-15.0	28.6	-13.6	-147.2	171.5	-24.3	N	N	P	P

Moc= cv. Moctezuma; Cid= cv. Cid; -N= nitrogen deficiency, -P= phosphorus deficiency, -K= potassium deficiency.

Table 4. Nutritional factors studied, petiole extract (PE) and leaf (lab) levels, levels of existing standards and established standards (DRIS) in two tomato cultivars. ([x]Scucuglia & Creste, 2014). Querétaro, UAQ, 2018.

Nutrient	N		P		K	
	mg L^{-1}	CV	mg L^{-1}	CV	mg L^{-1}	CV
PSA Treatments	550-1100	30	190-730	46	1500-1800	6
PSA Control	850-1420	22	720-870	8	1520-2150	14
	g kg^{-1}	CV	g kg^{-1}	CV	g kg^{-1}	CV
Lab Treatment	30-39	8	2-6	46	22-36	21
Lab Control	40-41	1	6-8	12	39-42	3
DRIS tomato[x]	29-44		4-7		35-51	

CV= coefficient of variation.

otherwise insufficient to satisfy the high demand of the new growth zones, as K is mainly required in the fruits (Marschner, 2011) (Table 1).

Colorimetric analysis of N, P, and K in tomato sap yielded values similar to those obtained in the laboratory (foliar analysis), with the values of the two approaches having correlation coefficients greater than 80% (Table 2). Such an approach can allow a producer to rapidly make decisions regarding nutritional imbalances. Colorimetry analysis of N, P, and K has been reported to achieve good results, similar to those obtained using standard laboratory methods (Hochmuth et al., 2004). For example, Peña-Fleitas et al. (2015) reported an R^2 of 0.88 for the correlation between the sap NO^{-3} content of greenhouse tomatoes and the N status in the plant. These observations suggest that colorimetric analysis of PWE is a good method for determining the status of N, P, and K in greenhouse tomato. In the present study, very strong relationships were observed between leaf N, P, and K contents and the corresponding contents in PE in the deficiency treatments; for example, R^2 values of 0.86 to 0.88 for N were obtained. Among the elements, P exhibited the strongest relationship (R^2= 0.90 to 0.97) between leaf and PE. These strong relationships were observed because the nutrients were extracted directly from conductive tissue and not from structural tissue. Compared with leaf analysis, PSA offers a more direct indication of the status of N, P, and K in tomato plants since leaf values of N, P and K are less variable than are PE values in response to element deficiencies in crops (Figure 1).

The DRIS method numerically describes those elements generating nutritional imbalances that can affect crop yield in the form of indices. A negative index indicates that an element is deficient, whereas a positive index indicates its excess; values near zero indicate element balance. Currently, it is difficult to determine how much the excess or deficiency of an element is causing variation in performance. Using the DRIS methodology with foliar analysis, induced deficiencies were confirmed for all treatments. However, in the control treatments of both cultivars, where there were no induced deficiencies, N deficiency was identified because it is impossible for all elements to be well balanced in the leaf such that the index values are zero. The absorption of nutrients by plants depends on genetic and environmental factors (Wang & Wu, 2015). This dependence is reflected in the variability of the indices between the control treatments, both using the traditional method (foliar analysis) and the one proposed in this research (PSA-DRIS). For example, for the Cid cultivar, a N index of -79.74 (PSA-DRIS) was obtained, whereas for Moctezuma, the corresponding value was -147.22. However, the DRIS method analyzes nutrients in pairs, ultimately determining which of them is in imbalance regardless of the cultivar used (Walworth & Sumner, 1987). In both cultivars, using PSA with the DRIS methodology to determine the nutritional imbalances of N, P and K yielded indices similar to those obtained using the conventional DRIS method (control treatments T7 and T8). However, in those treatments inducing deficiencies, PSA-DRIS

was only effective relative to DRIS in detecting N deficiencies in both cultivars and K deficiency in the Cid cultivar. In contrast, with the DRIS methodology, P excess was identified in 75% of the treatments (i.e., of all treatments, including the control treatments), whereas with the PSA-DRIS, P excess was identified in 100% of the treatments (Table 3).

The results of the foliar analysis in the laboratory are consistent with other studies (Scucuglia & Creste, 2014); however, the PSA yielded results with little variation, possibly reflecting the developmental stage of the plant. On average, the N deficiency identified by PSA in the Cid cultivar was 815 mg L^{-1}, whereas that for the Moctezuma

cultivar was 600 mg L^{-1}, well below the average value reported as sufficient (1050 mg L^{-1}) in tomato sap (Peña-Fleitas et al., 2015). These findings confirm that deficiency was induced by the treatments. Among the elements, P presented the greatest variation in both the laboratory analysis and the PSA (Table 4). The absolute NPK contents obtained in this study are comparable to the DRIS standards established by Scucuglia & Creste (2014) for both treatments and controls. However, there were lower CV values for the results of the control treatments than for those of the deficiency treatments with respect to the reported standards, especially for P (Table 4).

This study confirmed that equipments

such as colorimeters that can rapidly and economically measure NPK and other elements in petiole extract can detect nutrient deficiencies in tomato crops more rapidly than can conventional laboratory analyses.

The PSA method successfully detected 62.5% of the cases of deficiency identified via conventional DRIS analysis, and 100% agreement in index values of the controls was obtained between DRIS and PSA-DRIS.

More research is necessary to improve the detection of nutrient deficiencies in the PE of tomato using the PSA-DRIS methodology, since as shown in this study, each variety may respond differently during
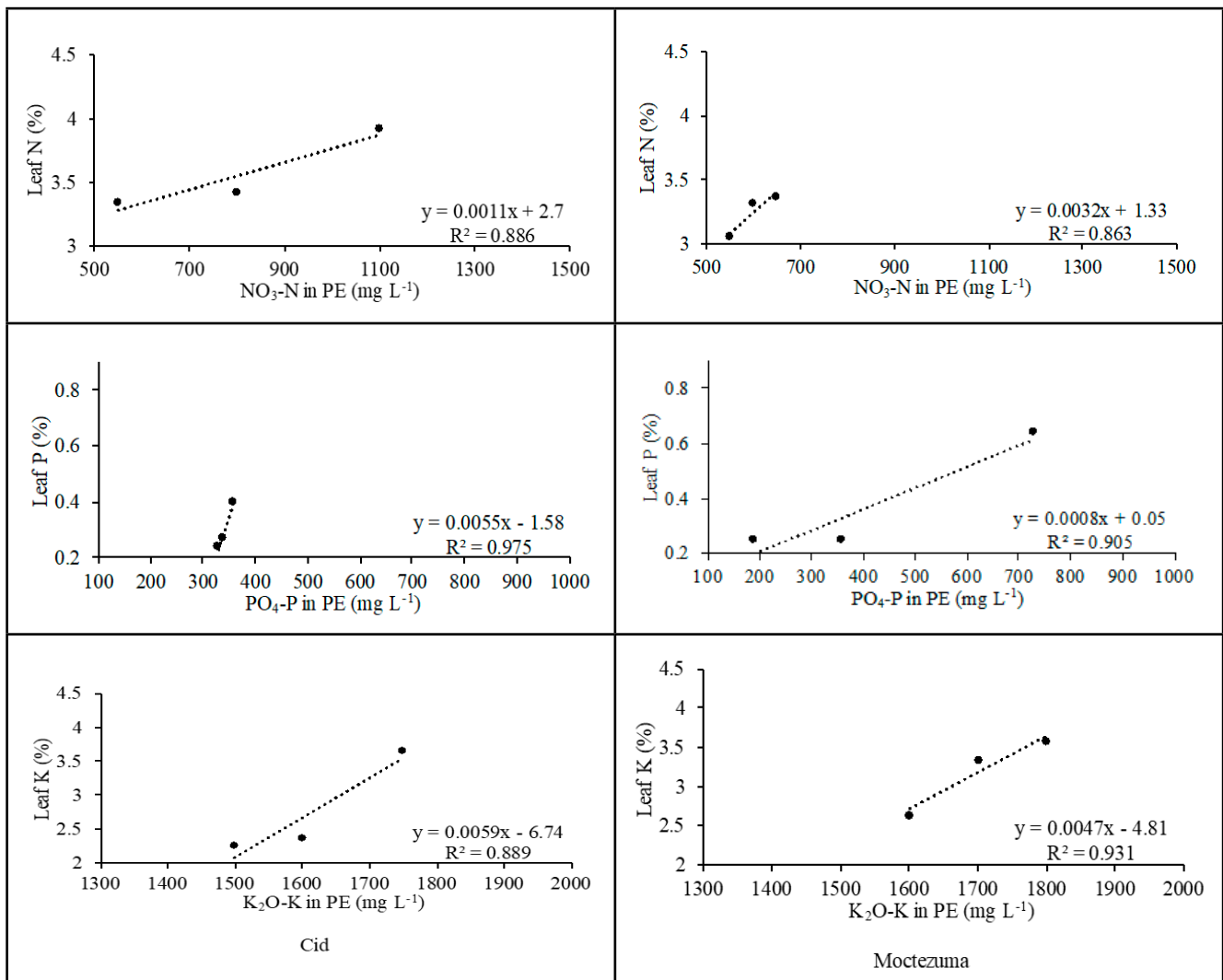

Figure 1. Relationships between leaf and petiole extract (PE) concentrations of nitrogen, phosphorus, and potassium in two tomato cultivars with induced NPK deficiencies. Querétaro, UAQ, 2018.

nutrient absorption, while conversely, environmental factors can influence such nutrient absorption.

ACKNOWLEDGMENTS

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