

## Compositional Nutrient Diagnosis In Nopal (*Opuntia ficus-indica*)<sup>♦</sup>

Rafael Magallanes-Quintanar<sup>1</sup>, Ricardo David Valdez-Cepeda<sup>2</sup>, Fidel Blanco-Macias<sup>2</sup>,  
Miguel Márquez-Madrid<sup>2</sup>, Raúl René Ruíz-Garduño<sup>2</sup>, Oscar Pérez-Veyna<sup>3</sup>,  
José Luis García-Hernández<sup>4</sup>, Bernardo Murillo-Amador<sup>4</sup>, José Dimas López-Martínez<sup>5</sup>,  
and Enrique Martínez-Rubín de Celis<sup>6</sup>

<sup>1</sup>Universidad Autónoma de Zacatecas, Unidad Académica de Medicina Veterinaria y Zootecnia,  
Programa de Doctorado en Ciencias Pecuarias. Apdo. Postal 11,  
CP 98500, Calera de V.R., Zacatecas, México

<sup>2</sup>Universidad Autónoma Chapingo, Centro Regional Universitario Centro-Norte, MCDRR.  
Apdo. Postal 196, CP 98001, Zacatecas, Zac. México  
Fax No. +52 492 9246147. E-mail: [vacrida@hotmail.com](mailto:vacrida@hotmail.com)  
Guest Researcher at the Universidad Autónoma de Zacatecas, Unidad Académica de Matemáticas.  
Calzada Solidaridad s/n, esquina con carretera a La Bufa. Zacatecas, Zac., México

<sup>3</sup>Universidad Autónoma de Zacatecas, Centro Universitario de Cómputo.  
Ramón López Velarde # 707, interior D. CP 98064, Zacatecas, Zac. México

<sup>4</sup>Centro de Investigaciones Biológicas del Noroeste, S.C. Apdo. Postal 128,  
CP 23090, La Paz, B.C.S., México

<sup>5</sup>Facultad de Agricultura y Zootecnia, Universidad Juárez del Estado de Durango.  
Apdo. Postal 142, CP 35000, Gómez Palacio, Dgo., México

<sup>6</sup>Instituto Tecnológico Agropecuario de la Laguna. Apdo. Postal 3-F,  
CP 3500, Torreón, Coah., México

### ABSTRACT

The appraisal of soil fertility and the assessment of plant mineral requirements are fundamental for crop management. This study was conducted to determine nutrient norms and to identify significant nutrient interactions in nopal (*Opuntia ficus-indica*). Preliminary compositional nutrient diagnosis (CND) norms were developed from a small database as means and standard deviations of row-centered log ratios ( $V_x$ ) of five nutrients (N, P, K, Ca, and Mg) and a filling value, R, which comprises all nutrients not chemically analyzed and quantified in nopal plants. Preliminary CND norms are:  $V_N^* = -1.13336 \pm 0.0766$ ,  $V_P^* = -2.26110 \pm 0.1093$ ,  $V_K^* = 0.36715 \pm 0.2329$ ,  $V_{Ca}^* = 0.37021 \pm 0.1047$ ,  $V_{Mg}^* = -0.7257 \pm 0.1413$ , and  $V_{R_5}^* = 3.38281 \pm 0.0833$ . These norms qualify yields higher than 35 kg pl<sup>-1</sup> (fresh matter of cladodes one-year old) registered in an experimental plot with a plant density of 10000 plants per hectare, and are associated to 0.97% N, 0.31% P, 4.47% K, 4.37% Ca, and 1.47% Mg. Four important nutrient interactions were evidenced through principal component analyses: Ca-Mg, Ca-N, Mg-N, and K-P.

**Key Words:** compositional nutrient diagnosis approach; nutrient interactions; nutrient norms

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## INTRODUCTION

Plant foods contain almost all of the mineral and organic nutrients established as essential for animal and human nutrition, as well as a number of unique organic phytochemicals that have been linked to the promotion of good health. Thus, knowledge to understand the physiological, biochemical, and molecular mechanisms that contribute to their transport, synthesis and accumulation in plants is fundamental to improve the nutritional quality of plants, with respect to both nutrient composition and concentration.

Since the 19th century, it is well known that plant growth is always limited by the first factor whose availability in the aerial and root environments start to limit plant growth. Soil fertility is among these governing factors, meaning mineral nutrients might be limiting plant growth. Therefore, the appraisal of soil fertility and the assessment of plant mineral requirements are fundamental for crop management. In this context, whole plant or plant-organ testing is widely used because it gives a direct measurement of the actual quantities of nutrients taken up by the crop. The leaf has been the most used organ for this purpose.

Approaches to diagnosing leaf nutrient status include the Critical Value Approach (CVA) (Bates, 1971), the Diagnosis and Recommendation Integrated System (DRIS) (Walworth and Sumner, 1987), and Compositional Nutrient Diagnosis (CND) (Parent and Dafir, 1992; Parent *et al.*, 1994). When selecting nutrient norms, a yield cutoff value is decided arbitrarily for defining a high-yield subpopulation. For CVA, the cutoff value is generally 90% to 95% of maximum yield while relating percentage yield to nutrient concentration (Ware *et al.*, 1982), assuming that all nutrients except the one being diagnosed are in sufficient, nonexcessive amounts. For DRIS and CND, the high-yield subpopulation is selected from a crop survey database. Walworth and Sumner (1987) proposed to consider variance ratios of nutrient expressions to discriminate between the subpopulations. However, no formal procedure was proposed to optimize the partition. Parent and Dafir (1992) expected that multivariate analysis could provide a means to define the high-yield subpopulation. Parent *et al.* (1994) proposed the  $\chi^2$  distribution function to define a CND threshold value for nutrient imbalance.

At the local level, small databases are available to define effective nutrient norms as related to yield target (Walworth *et al.*, 1988). Escano *et al.* (1981) pointed out that local calibration improved the accuracy of DRIS diagnosis. However, DRIS provides no generic approach to support local diagnosis of nutrient imbalance using small databases as the CND approach does it because of the chi-square distribution function support (Parent *et al.*, 1994).

Few studies considering tissue analysis in nopal (*Opuntia ficus-indica*) have been developed on the concentration of the nutrients in cladodes. In general, N tends to be more concentrated in young cladodes than in mature ones (Nobel, 1983). Also, the cultivated nopal plants tend to accumulate more Ca in their cladodes in comparison with most of the cultivated species. Ca accumulation becomes serious with the age of the tissue. Also, differences in chemical composition of cladodes among species of nopal have been reported.

Gathaara *et al.* (1989) reported optimal concentrations on dry matter basis of 1.16% N and 0.115% P in *O. engelmannii* in order to produce cactus pear. Nerd and Mizrahi (1993) pointed out that concentrations from 0.75% to 0.97% N in cladodes induce high yields of cactus pear in *O. ficus-indica*. In addition, Claessens and Wessels (1997) reported optimal yields of cactus pear of *O. ficus-indica* with concentrations of 0.94% to 0.96% N, 0.15% to 0.2% P, 1.5% K, 1.8% to 2.5% Ca, and 0.7% to 1.1% Mg in cladodes. It has been demonstrated clearly that, for example, the increase of N in the cladodes induces vegetative and floral bloom (Nerd *et al.*, 1991; Valdez-Cepeda *et al.*, 2002) and higher production of cactus pear (Nerd and Mizrahi, 1992). Also, Gathaara *et al.* (1989) reported that with very high

concentrations of N in the cladodes, the vegetative growth is increased and the production of cactus pear in *O. engelmannii* is reduced.

The indicated evidences allowed Valdez-Cepeda *et al.* (2002) to conclude that with the aims of production of cactus pear, the nitrogen requirements are lower than corresponding to production of nopalitos (vegetable) and cladodes for propagation and animal consumption. Also, in the last cases it is necessary to be cautious with respect to the nitrogen fertilization, mainly, since the nopal has the particularity to easily absorb and to accumulate nitrates in its nopalitos and cladodes, and food can reach toxic levels for people and animals that eat them.

The aims of this paper are i) to compute the preliminary CND norms for nopal (*Opuntia ficus-indica*) grown on the experimental field of the Centro Regional Universitario Centro-Norte of the Universidad Autónoma Chapingo within the Mexico's Zacatecas state; and ii) to identify significant nutrient interactions through principal component analyses taking into account the CND indexes.

This paper is organized as follows. In the next section we describe briefly the theory about the CND approach. After, the CND norms are developed for the case of study. In other section, results of principal components analyses are shown in order to identify important nutrient interactions. Conclusions are presented in the final section.

### THEORY ABOUT THE COMPOSITIONAL NUTRIENT DIAGNOSIS APPROACH

As indicated by Parent and Dafir (1992), plant tissue composition forms a  $d$  dimensional nutrient arrangement, *i.e.*, simplex ( $S^d$ ) made of  $d+1$  nutrient proportions including  $d$  nutrients and a filling value defined as follows:

$$S^d = [(N, P, K, \dots, R_d) : N > 0, P > 0, K > 0, \dots, R_d > 0, N + P + K + \dots + R_d = 100] \quad (1)$$

where 100 is the dry matter concentration (%);  $N, P, K, \dots$  are nutrient proportions computed as follows:

$$R_d = 100 - (N + P + K + \dots) \quad (2)$$

The nutrient proportions become scale invariant after they have divided by geometric mean ( $G$ ) of the  $d + 1$  components including  $R_d$  (Aitchinson, 1986) as follows:

$$G = [N \cdot P \cdot K \cdot \dots \cdot R_d]^{\frac{1}{d+1}} \quad (3)$$

Row-centered log ratios are computed as follows:

$$V_N = \ln\left(\frac{N}{G}\right), V_P = \left(\frac{P}{G}\right), V_K = \left(\frac{K}{G}\right), \dots, V_{R_d} = \left(\frac{R_d}{G}\right) \quad (4)$$

and

$$V_N + V_P + V_K + \dots + V_{R_d} = 0 \quad (5)$$

where  $V_X$  is the CND row-centered log ratio expression for nutrient  $X$ . This operation is a control to insure that  $V_X$  computations have been conducted properly. By definition, the sum of tissue components is 100% (Eq. [1]), and the sum of their row-centered log ratios, including the filling value, must be zero (Eq. [5]).

After this stage, it is necessary to iterate a partition of the database between two subpopulations using the Cate-Nelson procedure once the observations have been ranked in a decreasing yield order (Khiari et al., 2001). In the first partition, the two highest yield values form one group, and the remainder of yield values forms another group; thereafter, the three highest yield values form the other. This process is repeated until the two lowest yield values forms one group, and the remainder of yield values forms the other. At each iteration, the first subpopulation comprises  $n_1$  observations, and the second comprises  $n_2$  observations for a total of  $n$  observations ( $n = n_1 + n_2$ ) in the whole database.

For the two subpopulations obtained at each iteration, one must compute the variance of CND  $V_X$  values. Then the variance ratio for component  $X$  can be estimated as follows:

$$f_i(V_x) = \frac{\text{Variance of } V_x \text{ } n_1 \text{ observations}}{\text{Variance of } V_x \text{ } n_2 \text{ observations}} \quad (6)$$

where  $f_i(V_x)$  is the ratio function between two subpopulations for nutrient  $X$  at the  $i$ th iteration ( $i = n_i - 1$ ) and the  $V_X$  is the CND row-centered log ratio expression for nutrient  $X$ . The first variance ratio function computed for the two highest yields is put on the same line as the highest yield, thus leaving three empty bottom lines.

The cumulative variance ratio function is the sum of variance ratios at the  $i$ th iteration from the top. The cumulated variance ratios for a given iteration is computed as a proportion of total sum of variance ratios across all iterations to compare the discrimination power of the  $V_X$  between low-yield and high-yield subpopulations on a common scale. So, the cumulative variance ratio function  $F_i^C(V_X)$  can be computed as follows:

$$F_i^C(V_X) = \left[ \frac{\sum_{i=1}^{n_1-1} f_i(V_X)}{\sum_{i=1}^{n_1-3} f_i(V_X)} \right] [100] \quad (7)$$

where  $n_1-1$  is partition number and  $n$  is total number of observations ( $n_1 + n_2$ ). The denominator is the sum of variance ratios across all iterations, and thus is a constant for nutrient  $X$ .

The cumulative function  $F_i^C(V_X)$  related to yield ( $Y$ ) shows a cubic pattern:

$$F_i^C(V_X) = aY^3 + bY^2 + cY + d \quad (8)$$

The inflection point is the point where the model shows a change in concavity. It is obtained by deriving Eq. [8] twice:

$$\frac{\partial F_i^C(V_X)}{\partial Y} = 3aY^2 + 2bY + C \quad (9)$$

$$\frac{\partial^2 F_i^C(V_X)}{\partial Y^2} = 6aY + 2b \quad (10)$$

The inflection point is then obtained by equating the second derivative (Eq. [10]) to zero. Thus the solution for the yield cutoff value is  $-b/3a$ .

The highest yield cutoff value across nutrient expressions can be selected to ascertain that minimum yield target for a high-yield subpopulation will be classified as high yield whatever the nutrition expression.

In this way, the CND norms can be calculated using the means and standard deviations corresponding to the row-centered log ratios  $V_X$  of  $d$  nutrients for the high-yield specimens, that is,  $V_N^*$ ,  $V_P^*$ ,  $V_K^*$ , ...,  $V_R^*$  and  $SD_N^*$ ,  $SD_P^*$ ,  $SD_K^*$ , ...,  $SD_R^*$ , respectively.

Once the CND norms are developed, they can be validated by using an independent database. They also can be used for diagnostic purposes as follows:

$$I_N = \frac{(V_N - V_N^*)}{SD_N^*}, I_P = \frac{(V_P - V_P^*)}{SD_P^*}, I_K = \frac{(V_K - V_K^*)}{SD_K^*}, \dots, I_{R_d} = \frac{(V_{R_d} - V_{R_d}^*)}{SD_{R_d}^*} \quad (11)$$

where  $I_N, \dots, I_{R_d}$  are the CND indices.

Additivity or independence among compositional data is ascertained using row-centered log ratio transformation (Aitchison, 1986). The CND indices as defined by Eq. [11] are standardized and linearized variables as dimensions of a circle ( $d + 1 = 2$ ), a sphere ( $d + 1 = 3$ ), or a hypersphere ( $d + 1 > 3$ ) in a  $d + 1$  dimensional space. The CND nutrient imbalance index of a diagnosed specimen is its CND  $r^2$  and is computed by:

$$r^2 = I_N^2 + I_P^2 + I_K^2 + \dots + I_{R_d}^2 \quad (12)$$

Its radius,  $r$ , computed from the CND nutrient indices, thus characterizes each specimen. The sum of  $d + 1$  squared independent, unit-normal variables produces a new variable having a  $\chi^2$  distribution with  $d + 1$  degrees of freedom (Ross, 1987). Because CND indices are independent, unit-normal variables, the CND  $r^2$  values must have a  $\chi^2$  distribution function. This is why it is recommended that the highest yield cutoff value (highest discrimination power) among  $d + 1$  nutrient computations be retained to calculate the proportion of the low-yield subpopulation below yield cutoff used as critical value for the  $\chi^2$  cumulative distribution function. As defined by Eqs. [11] and [12], the closer to zero that CDN indices are, and thus the CND  $r^2$  or  $\chi^2$  values are, the higher the probability to obtain a high yield.

## THE CND NOPAL (*Opuntia ficus-indica*) CASE STUDY

### Data

This study is based on data acquired from a field experiment to test three fertilization treatments and three varieties of *Opuntia ficus-indica* ('Jalpa', 'Villanueva' and 'Copena V1'). Within the experimental plot, a plant density of 10 000 plants per hectare was used. We are considering data from 36 plants (12 of each variety). Data corresponds to the concentration of N, P, K, Ca, and Mg in cladodes, and cladodes fresh

matter. All data are associated to one-year cladodes harvested from two-year-old plants having the same structure through formation-pruning practice. The cut cladodes were growing on nine cladodes at the second level from the mother cladode, from March 2001 to February 2002. Concentration of N was determined at the Laboratory of the Centro de Investigaciones Biológicas del Noroeste, La Paz, Baja California, México, through its conventional approaches after acid digestion of the dry tissue samples: N by vapor efflux, P by reduction with molibdo-vanadate, and K, Ca, and Mg by spectrophotometric techniques.

### The Compositional Nutrient Diagnosis Norms for Simplex S<sup>5</sup>

All the following calculations were performed using Microsoft Excel 2000 software (Microsoft Corp., 2000), using the mineral composition and fresh matter of one-year cladodes data.

The S, *i.e.*, six-dimensional ( $d + 1$ ) simplex comprised the five nutrients N, P, K, Ca, and Mg and the filling value R. R values were estimated using Eq. [1].

Nutrient concentrations were transformed into CND row-centered log ratios  $V_N$ ,  $V_P$ ,  $V_K$ ,  $V_{Ca}$ , and  $V_{R_d}$  through Eqs. [2] through [4]. We used Eq. [6] to estimate the  $F_i^C(V_X)$  values. The cutoff yield between the low- and high-yield subpopulations was determined after examining the five cubic cumulative variance ratio functions  $F_i^C(V_N)$ ,  $F_i^C(V_P)$ ,  $F_i^C(V_K)$ ,  $F_i^C(V_{Ca})$ ,  $F_i^C(V_{Mg})$ , and  $F_i^C(V_R)$  (Figure 1).

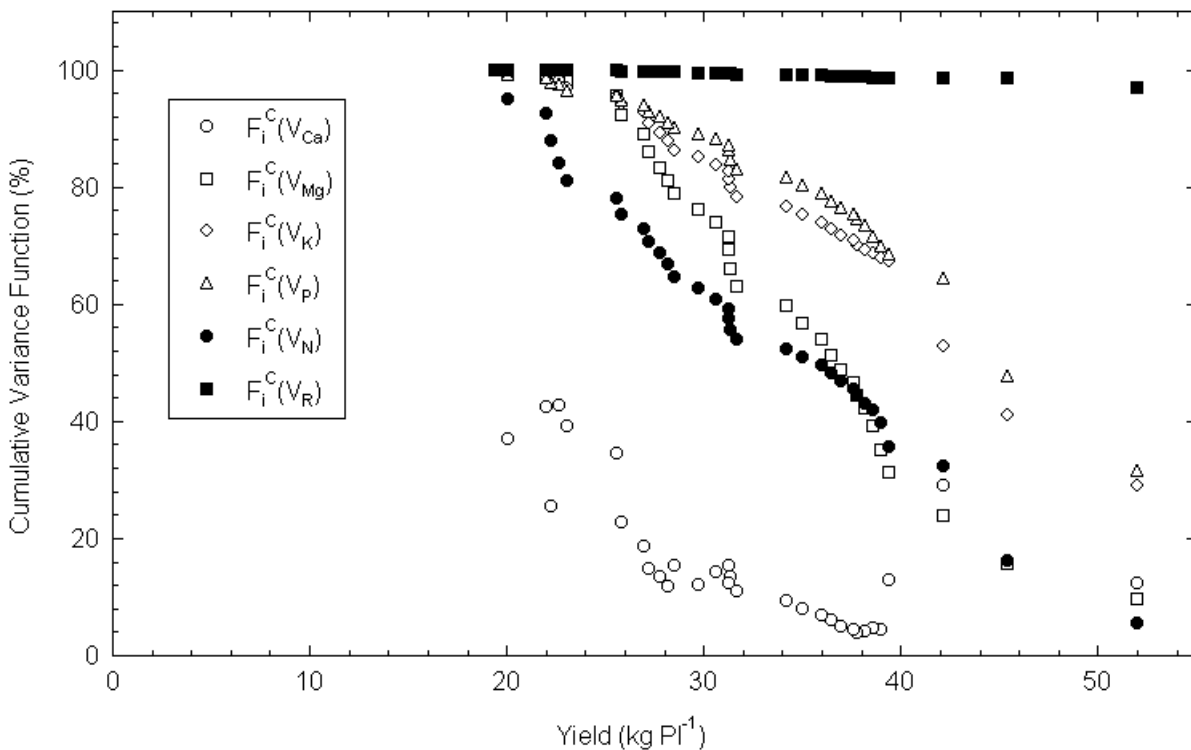


Figure 1. Relations between yield (fresh weight) in nopal and the functions of proportion of accumulated variance to consider the inflection point or division between subpopulation of high and low yields

The yields (kg PI<sup>-1</sup>) at inflection points of the cubic functions, computed by setting the second derivative to zero, were 37.90 kg PI<sup>-1</sup> for  $F_i^C(V_N)$ , 111.50 kg PI<sup>-1</sup> for  $F_i^C(V_P)$ , 195.71 kg PI<sup>-1</sup> for  $F_i^C(V_K)$ , 45.04 kg PI<sup>-1</sup> for  $F_i^C(V_{Ca})$ , 34.85 kg PI<sup>-1</sup> for  $F_i^C(V_{Mg})$  and 26.00 kg PI<sup>-1</sup> for  $F_i^C(V_R)$  (Table 1). The cubic model adjusted significantly in all the cases. Therefore, the decision was taken to indicate the yield of reference to 35 kg PI<sup>-1</sup> computed as the mean of valid inflection points, considering that the points of inflection for  $F_i^C(V_P)$  and  $F_i^C(V_K)$  are out of context. In this way, 12 observations (the third part) correspond to the subpopulation of high yields and 24 to the one of low yields.

Table 1. Yield (Fresh Matter) of Nopal at Inflection Points of Cumulative Variance Functions for Row-Centered Log Ratios [ $F_i^C(V_X)$ ] in the Survey Population (n=36)

Nutrient	$F_i^C(V_X) = aY^3 + bY^2 + cY + d$	R <sup>2</sup>	Yield at inflection point (-b/3a) (kg PI <sup>-1</sup> )
N	-0.0017y <sup>3</sup> + 0.1933y <sup>2</sup> - 9.7812y + 227.79	0.987	37.90
P	0.0002y <sup>3</sup> - 0.0669y <sup>2</sup> + 2.0378y + 83.779	0.992	111.50
K	7E-05y <sup>3</sup> - 0.0411y <sup>2</sup> + 0.3415y + 110.39	0.983	195.71
Ca	-0.0035y <sup>3</sup> + 0.4729 y <sup>2</sup> - 20.355y + 293.62	0.750	45.04
Mg	0.0057y <sup>3</sup> - 0.596y <sup>2</sup> + 16.422y - 34.252	0.992	34.85
R	-5E-05y <sup>3</sup> + 0.0039y <sup>2</sup> - 0.1659y + 102.21	0.970	26.00

The CND norms as means and standard deviations of  $V_N$ ,  $V_P$ ,  $V_K$ ,  $V_{Ca}$ ,  $V_{Mg}$ , and  $V_{Rd}$  for the high-yield subpopulation (>35 kg PI<sup>-1</sup>) Corresponding optimum ranges of nutrients for nopal cladode production are presented in Table 2.

Table 2. Norms of Compositional Nutrient Diagnosis (CND) and Optimum Ranges (mean ± standard deviation, SD) of Nutrients for Cladode Production With a Yield Cutoff Value of Reference of 35 kg Fresh Matter PI<sup>-1</sup>

Row-centered log ratio	CND Norm		Nutrient	Range (Mean ± SD)	
	Mean	SD		Mean (%)	SD (%)
$V_N^*$	-1.13	0.076	N	0.972	0.111
$V_P^*$	-2.26	0.109	P	0.314	0.034
$V_K^*$	0.367	0.233	K	4.470	1.048
$V_{Ca}^*$	0.370	0.105	Ca	4.369	0.460
$V_{Mg}^*$	-0.726	0.141	Mg	1.469	0.232
$V_R^*$	3.383	0.083			
$\Sigma V_X$	0	-			

CND nutrient indices  $I_N$ ,  $I_P$ ,  $I_K$ ,  $I_{Ca}$ ,  $I_{Mg}$ , and  $I_{Rd}$  were estimated using Eq. [11]; and CND  $r^2$  values were computed through Eq. [12]. The CND  $r^2$  values were distributed like  $\chi^2$  values ( $R^2 > 0.999$ ,  $P < 0.001$ ) (Figure 2). We found that 70% of the observations, *i.e.*, 100 minus 30% of the population, were below yield cutoff of 35.0 kg PI<sup>-1</sup>, and the corresponding  $\chi^2$  value with 6 df was 3.8. Thereby, the critical  $\chi^2$

value of 3.8 is the maximum  $\chi^2$  value for qualifying a sample in the high-yield subpopulation. Readers have got to keep in mind these values (70% and 3.8 from Figure 2) when validating the preliminary CND norms, because the independent dataset ought to be characterized by similar values.

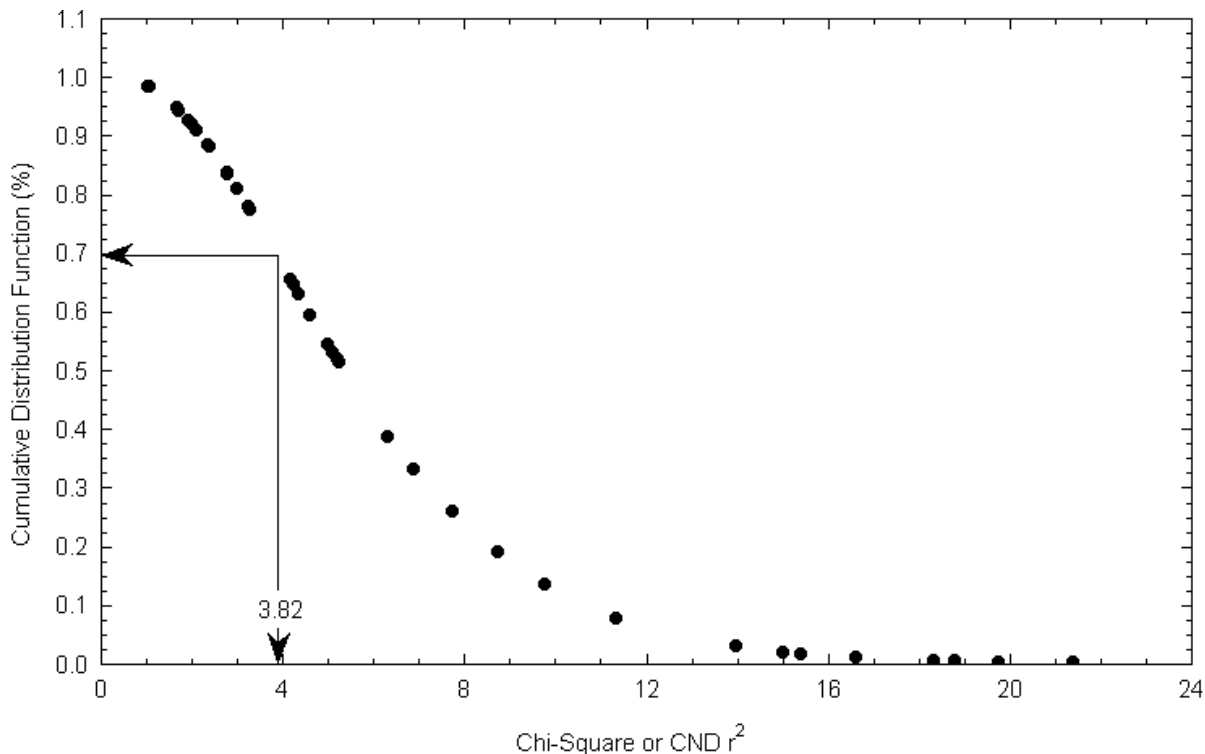


Figure 2. The  $\chi^2$  Distribution Function With 6 df to Obtain Theoretical  $\chi^2$  Threshold

The range for N pointed out in Table 2 for cladode production agrees with these reported by Nerd and Mizrahi (1992) and Claessens and Wessels (1997) for *O. ficus-indica* with the aim of fruit production. However, the found range does not correspond to the mean N concentration in cladodes of *O. engelmannii* consigned by [Gathaara et al. \(1989\)](#); thus, this disagreement could be due to the difference of vegetative material.

The range for P in Table 2 is higher than the concentration reported by [Gathaara et al. \(1989\)](#) and the range pointed out by [Claessens and Wessels \(1997\)](#), and there is a similar situation for K, Ca, and Mg when considering the concentrations reported by Claessens and Wessels (1997).

## NUTRIENT INTERACTIONS THROUGH PRINCIPAL COMPONENT ANALYSES

Principal component analysis (PCA) was conducted on CND indexes for three cases: considering the whole dataset (36 observations), considering the high-yielding subpopulation (12 observations), and considering the low-yielding subpopulation (24 observations). PCA was performed using factor analysis module of the Statistica package version 4.2 (StatSoft Inc., 1998). Interpretation of the principal components (PCs) was aided by inspection of the factor-loading matrix extracted from a varimax rotated procedure performed to obtain maximum relationships among indexes and contribution of them to the



PCs (Ovalles and Collins, 1988; Gutiérrez-Acosta *et al.*, 2002). This is a matrix of correlations between the PCs and the CND indices. The correlations between some of the CND indices and the PCs became high or low; the interpretation of the PC's structures was then easier.

Two PCs explain almost 72% of the total variance in the case of the whole dataset (Table 3). On the other hand, two PCs in the cases of high-yield and low-yield subpopulations explain 78% and 72%, (Table 3). It is appreciated that the structure of the two PCs differ in all three cases.

For the PC1 in the whole dataset, I Ca and I Mg define the structure, and the positive interaction between Ca and Mg is also important. For the PC2, I K and I R are the significant factors in the structure, and the important interaction between K and R is negative.

For the high-yield dataset, PC1 was positively correlated with I Ca and I Mg and negatively correlated with I N (Table 3). It suggests a positive interaction between Ca and Mg, and two negative interactions: Ca-N and Mg-N. PC2 was negatively correlated with I K and positively correlated with I P and I R, meaning a positive interaction between P and R, and two negative interactions: K-P and K-R.

The case of the low-yield subpopulation highlights a PC1 negatively correlated with I R and positively correlated with I K (Table 3). There an important negative interaction between R and K was evidenced. Moreover, PC2 was negatively correlated with I Mg and positively correlated with I N, suggesting a negative interaction between N and Mg.

Table 3. Loadings or Correlations Between the Row-Centered Log Ratios and the First Two Principal Components (PCs) for the Whole Dataset (36 Observations), High-Yield Subpopulation (12 Observations), and Low-Yield Subpopulation (24 Observations)  
Extracted from Varimax-Normalized Matrices

CND index	Whole Dataset (n=36)		High-Yield Dataset (n=12)		Low-Yield Dataset (n=24)	
	PC1	PC2	PC1	PC2	PC1	PC2
I N	0.511	0.029	<b>-0.737</b>	0.040	0.065	<b>0.821</b>
I P	0.599	-0.653	-0.459	<b>0.745</b>	-0.677	0.574
I K	0.415	<b>0.819</b>	-0.575	<b>-0.756</b>	<b>0.749</b>	-0.026
I Ca	<b>-0.884</b>	0.198	<b>0.959</b>	0.217	0.528	-0.669
I Mg	<b>-0.910</b>	0.058	<b>0.914</b>	0.008	0.283	<b>-0.808</b>
I R	0.293	<b>-0.821</b>	0.129	<b>0.813</b>	<b>-0.896</b>	0.186
Explained Variance	2.489	1.815	2.856	1.837	2.187	2.140
Proportion of Total	0.415	0.302	0.476	0.306	0.364	0.357
Cumulative Variance	0.415	0.717	0.476	0.782	0.364	0.721

Values in boldface are the dominant in the eigenvector loadings by setting the level of significance at approximately 0.7.

Remarkably, four interactions are strongly evidenced: Ca-Mg, Ca-N, Mg-N, and K-P. The first interaction disagrees with previous findings because, commonly, Ca<sup>2+</sup> is strongly competitive with Mg<sup>2+</sup> in substrates and often result in increased leaf-Ca along with a marked reduction in leaf Mg (Ruiz *et al.*, 1997; Grattan and Grieve, 1999). Recently, Appenroth *et al.* (1999) confirmed a competitive ion antagonism between Ca<sup>2+</sup> and Mg<sup>2+</sup>.

It has been reported that as the NH<sub>4</sub><sup>+</sup>/NO<sub>4</sub><sup>+</sup> ratio increased, plants accumulated less Ca<sup>2+</sup> in their leaves (Grattan and Grieve, 1999), thus explaining the negative interaction between N and Ca.

Little progress has been reached on K-P interactions. However, Reneau *et al.* (1983) cited by Sumner and Farina (1986) have demonstrated that K-P interaction is important in forage sorghum production. Also, Sumner and Farina (1986) pointed out that the balance between K and P is important.

To identify which of the evidenced interactions is more important to differentiate the subpopulations, an *F* test was performed for each interaction (Table 4). None was significant at the 0.95 level. However, the interaction between Ca and Mg reached the highest *F* value ( $F = 2.246$ ), suggesting that it is the most important interaction defining the subpopulations of higher and lower yields of fresh matter of nopal in this case study. This result agrees with the idea that cultivated young plants of nopal tend to accumulate more Ca in their cladodes than other cultivated trees. Therefore, research on this and other nutrient interactions occurring in *Opuntia* spp. must be carried out in the near future in order to understand their role as factors limiting plant growth.

Table 4. Basic Statistics of the High-Yield and Low-Yield Subpopulations, and *F* Tests for the Important Nutrient Interactions

Nutrient proportion	Statistic	High Yield	Low Yield	<i>F</i> ratio
		(n = 12)	(n = 24)	
Ca/Mg	Mean	2.9999	2.9973	2.246
	SD	0.2293	0.3437	
Ca/N	Mean	4.5554	4.4875	1.316
	SD	0.7513	0.8620	
K/P	Mean	14.2808	13.5934	1.485
	SD	3.0399	2.4948	
K/R	Mean	0.0507	0.0496	1.844
	SD	0.0124	0.0091	

## CONCLUSIONS

The preliminary compositional nutrient diagnosis (CND) norms expressed as row-centered log ratios (means  $\pm$  standard deviations) for  $d = 5$  nutrients in a high-yield subpopulation producing more than 35 kg pl<sup>-1</sup> of nopal cladodes we propose are:  $V_N^* = -1.13336 \pm 0.0766$ ,  $V_P^* = -2.26110 \pm 0.1093$ ,  $V_K^* = 0.36715 \pm 0.2329$ ,  $V_{Ca}^* = 0.37021 \pm 0.1047$ ,  $V_{Mg}^* = -0.7257 \pm 0.1413$ , and  $V_{R_5}^* = 3.38281 \pm 0.0833$ .

The corresponding optimum ranges (means  $\pm$  standard deviations) of nutrients for cladode production with a yield cutoff value of reference of 35 kg pl<sup>-1</sup> are:  $N = 0.97191 \pm 0.1108\%$ ,  $P = 0.31458 \pm 0.340\%$ ,  $K = 4.4699 \pm 0.10478\%$ ,  $Ca = 4.36874 \pm 0.4605\%$ , and  $Mg = 1.46936 \pm 0.2321\%$ .

The following nutrient interactions were evidenced: Antagonisms between Ca and N, Mg and N, and K and P; and a positive interaction between Ca and Mg.

Research workers interested in this topic must take into consideration that the proposed norms are in their preliminary stage and, when attempting the validation of such norms, it is necessary to use an independent database.

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