ELSEVIER

Available online at www.sciencedirect.com
ScienceDirect

Environmental and Experimental Botany 58 (2006) 244–252

Environmental and Experimental Botany

www.elsevier.com/locate/envexpbot

Preliminary compositional nutrient diagnosis norms in *Aloe vera* L. grown on calcareous soil in an arid environment

José L. García-Hernández [a,*], Ricardo D. Valdez-Cepeda [b,c], Bernardo Murillo-Amador [a], F. Alfredo Beltrán-Morales [d], Francisco H. Ruiz-Espinoza [d], Ignacio Orona-Castillo [e], Arnoldo Flores-Hernández [f], Enrique Troyo-Diéguez [a]

[a] *Centro de Investigaciones Biológicas del Noroeste, SC (CIBNOR, SC), Programa de Agricultura en Zonas Áridas, Mar Bermejo #195, Col. Playa Palo Santa Rita, La Paz, BCS, CP 23090, Mexico*
[b] *Universidad Autónoma Chapingo, Centro Regional Universitario Centro Norte, Apartado Postal 196, Zacatecas, Zac., CP 98001, Mexico*
[c] *Universidad Autónoma de Zacatecas, Unidad Académica de Matemáticas, Paseo Solidaridad esquina con Carretera a la Bufa s/n, Zacatecas, Zac., CP 98060, Mexico*
[d] *Universidad Autónoma de Baja California Sur, La Paz, BCS, Mexico*
[e] *Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Centro Nacional de Investigación Disciplinaria en la Relación Agua, Suelo, Planta y Atmósfera, Cd. Lerdo, Dgo., Mexico*
[f] *Universidad Autónoma Chapingo, Unidad Regional de Zonas Áridas, Bermejillo, Dgo., Mexico*

Received 3 January 2005; received in revised form 13 June 2005; accepted 30 September 2005

Abstract

This study calculated the compositional nutrient diagnosis norms of *Aloe vera* L., and also identified significant nutrient interactions of this crop growing in an irrigated calcareous desert soil. The soil showed high heterogeneity within its chemical properties. For statistical analysis, 64 foliar composite samples from healthy plants were used. Preliminary compositional nutrient diagnosis norms were developed using a cumulative variance ratio function and the chi-square distribution function. Means and standard deviations are given of row-centered log ratios V_X of five nutrients (N, P, K, Ca, and Mg) and a filling value R, which included all nutrients not chemically analysed. Preliminary compositional nutrient diagnosis norms are: $V^*_{\mathrm{N}} = -1.033 \pm 0.105$, $V^*_{\mathrm{P}} = -2.617 \pm 0.142$, $V^*_{\mathrm{K}} = -0.041 \pm 0.201$, $V^*_{\mathrm{Ca}} = 0.692 \pm 0.168$, $V^*_{\mathrm{Mg}} = -0.7 \pm 0.128$, and $V^*_{R_5} = 3.699 \pm 0.104$. These compositional nutrient diagnosis norms for fresh matter, providing more than 136.67 t ha^{-1}, are associated with the following foliar concentrations: 8.13 g N kg^{-1}, 1.68 g P kg^{-1}, 22.39 g K kg^{-1}, 45.42 g Ca kg^{-1}, and 11.33 g Mg kg^{-1}. Through the principal component analysis of the CND indexes, the positive interactions P–K and Ca–Mg, and the negative interactions P–Ca, P–Mg, K–Ca, K–Mg, and N–R were identified. By using Pearson correlations, the same interactions were identified plus the following: negative P–R and K–R, and positive Ca–R and Mg–R. Estimated t- and F-values and their corresponding probability levels indicated that N/R antagonism, Ca/R and Mg/R synergisms, and nutrients N, Ca and R were the factors discriminating high- from low-yielding subpopulations.

Keywords: Nutrient norms; Nutrient interactions; Plant nutrition; *Aloe vera*

1. Introduction

Arid environments are found in low-latitude deserts approximately between 18° and 28° in both hemispheres. The dry arid desert is a true desert climate, and covers 12% of the Earth's land surface (Strahler and Strahler, 1984). In a global range, this climate prevails in southwestern United States and northern Mexico, Argentina, North Africa, South Africa, and central part of Australia. By this way, it is very difficult to generate economical activities for all people, mostly rural people, which face a big number of constraints for agriculture. Hence, in order to support their main activity, there is a need to define new productive alternatives. In this context, a crop species with high potential for arid and semi-arid areas

---

* Corresponding author. Tel.: +52 612 123 8484; fax: +52 612 123 8525.
E-mail addresses: jlgarcia04@cibnor.mx, luis_garher2000@yahoo.com.mx (J.L. García-Hernández).

0098-8472/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2005.09.001

inside these countries is *Aloe vera*. A remarkable advantage of this plant is its crassulacean acid metabolism (CAM), a specialized mode of photosynthetic carbon assimilation that has evolved in response to exceptional environmental conditions (Borland and Taybi, 2004).

A. vera is widely recognized for containing a number of unique organic phytochemicals in its leaves that favour human health. In the most recent years, many studies have been carried out in order to evaluate its role in the control or cure of many human diseases, i.e., for controlling gastric injuries (Yusuf et al., 2004), for anti-microbial activity (Moody et al., 2004), for defending against hippocampus and cerebral cortex oxidative damage (Parihar et al., 2004), for curing acne (Orafidiya et al., 2004), for anti-inflammatory activity (Vazquez et al., 1996), and other medical characters (Adusumilli et al., 2004). For these reasons, *A. vera* is a potentially valuable new medicinal crop in arid lands. Therefore, understanding the physiological, biochemical, and molecular mechanisms that contribute to nutrient absorption, transport, synthesis, and accumulation in the species, under many environmental conditions, is essential to improve the nutritional value of the plant in terms of nutrient composition and concentration (Mattos et al., 2003).

Several approaches can be used to diagnose foliar nutrient status, i.e., critical value approach (CVA, Bates, 1971), 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 (Khiari et al., 2001). The cutoff value for CVA is generally 90–95% of maximum yield while relating percentage yield to nutrient concentration (Ware et al., 1982), considering that all nutrients, except the one being diagnosed, are in sufficient, non-excessive amounts. For DRIS and CND approaches, the high-yield subpopulation is selected from a crop survey database. In the case of DRIS, Walworth and Sumner (1987) proposed variance ratios of nutrient expressions to discriminate between the high- and low-yield subpopulations; however, no formal procedure was proposed to optimize the partition. For example, Navvabzdeh and Malakouti (1993) divided 50 yields into high- and low-yielding groups using yields equal to or greater than 30 t ha^{-1} as the dividing criterion to compute DRIS norms for potato in calcareous soils of Iran. On the other hand, Parent and Dafir (1992) indicated that multivariate analysis provide a means to define a high-yield subpopulation. Parent and Dafir (1992) and Parent et al. (1994) proposed the chi-square distribution function to define a CND threshold value for nutrient imbalance when relating yield and the cumulative variance ratio function for each nutrient. The CND approach has a robust mathematical basis to define a minimum yield target that is useful for discriminating between high- and low-yield subpopulations.

Accumulating a large database is obviously time consuming and expensive (Alegre et al., 2003) when databases are often of limited size (Khiari et al., 2001). 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, because of the support of the chi-square distribution function (Parent et al., 1994).

Scientific literature now presents a great number of studies about the therapeutic properties of *A. vera*; however, the lack of studies on mineral nutrition and the diagnosis of *A. vera* nutrient status is remarkable.

In this study, preliminary CND norms were calculated for *A. vera* grown in a calcareous desert soil near La Paz, Baja California Sur, Mexico, and identified significant nutrient interactions through principal component analyses, taking into account CND indices.

2. Materials and methods

2.1. Theory of the CND approach

In order to calculate the preliminary compositional nutrient diagnosis norms, we used the CND approach, which has been described in Khiari et al. (2001). The approach is based in the plant tissue composition, which 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 R (Parent and Dafir, 1992). The theory is applied as follow:

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

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

$$R_d = 100 - (\mathrm{N} + \mathrm{P} + \mathrm{K} + \cdots). \quad (2)$$

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

$$G = [\mathrm{N} \cdot \mathrm{P} \cdot \mathrm{K} \cdots R_d]^{1/d+1}. \quad (3)$$

Row-centered log ratios are computed as:

$$V_\mathrm{N} = \ln\left(\frac{N}{G}\right),\ V_\mathrm{P} = \ln\left(\frac{P}{G}\right),\ V_\mathrm{K} = \ln\left(\frac{K}{G}\right), \ldots, V_{R_d} = \ln\left(\frac{R_d}{G}\right), \quad (4)$$

and

$$V_\mathrm{N} + V_\mathrm{P} + V_\mathrm{K} + \cdots + V_{R_d} = 0, \quad (5)$$

where V_X is the CND row-centered log ratio expression for nutrient X. The sum of tissue components is 100%, as in Eq.

(1), and the sum of their row-centered log ratios, including the filling value must be zero, as in Eq. (5).

Thereafter, the database is partitioned 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 (group A) and the remainder of yield values forms another group (group B); thereafter, the three highest yield values form the group A. This process is repeated until the two lowest yield values form group B, and the remainder of yield values forms the group A. At each iteration, the group A comprises n_1 observations, and the group B comprises n_2 observations for a total of n observations ($n = n_1 + n_2$) in the whole database. For the two subpopulations, one must compute the variance of the CND V_X values. Then the variance ratio for component X can be estimated as:

$$f_i(V_X) = \frac{\text{variance of } V_X n_1 \text{ observations}}{\text{variance of } V_X n_2 \text{ observations}}, \tag{6}$$

where $f_i(V_X)$ is the variance ratio function between two subpopulations for nutrient X at the ith 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, and so on, thus leaving three empty bottom lines.

The cumulative variance ratio function is the sum of variance ratios at the ith iteration from 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- and high-yield subpopulations on a common scale. The cumulative variance ratio function $F_i^{\mathrm{C}}(V_X)$ can then be computed as:

$$F_i^{\mathrm{C}}(V_X) = \left[\frac{\sum_{i=1}^{n-1} f_i(V_X)}{\sum_{i=1}^{n-3} f_i(V_X)}\right] \cdot [100], \tag{7}$$

where $n-1$ is the partition number and n is the total number of observations ($n_1 + n_2$). The denominator is the sum of variance ratios across all iterations, and is a constant for nutrient X. The cumulative function $F_i^{\mathrm{C}}(V_X)$ related to yield (Y) shows a cubic pattern, and the inflection point is the point where the model shows a change in concavity, it is obtained by the second derivation:

$$F_i^{\mathrm{C}}(V_X) = aY^3 + bY^2 + cY + d. \tag{8}$$

$$\frac{\partial^2 F_i^{\mathrm{C}}(V_X)}{\partial Y^2} = 6aY + 2b. \tag{9}$$

The inflection point is then obtained by equating the second derivative of Eq. (9) to 0. Therefore, the solution for the yield cutoff value is *−b/3a*. The highest yield cutoff value across nutrient expressions can be selected to determine what minimum yield target for a high-yield subpopulation will be classified as high yield, whatever the nutrition expression. CND norms are computed using means and standard deviations corresponding to the row-centered log ratios V_X of d nutrients for high-yield specimens, that is, V_{N}^*, V_{P}^*, $V_{\mathrm{K}}^*, \ldots, V_{R}^*$, and $\mathrm{S.D.}_{\mathrm{N}}^*$, $\mathrm{S.D.}_{\mathrm{P}}^*$, $\mathrm{S.D.}_{\mathrm{K}}^*, \ldots, \mathrm{S.D.}_{R}^*$, respectively.

Once CND norms have been developed, one can be able to estimate CND nutrient indexes as well as to validate those norms using an independent database. Validation of CND norms have been reported by Parent and Dafir (1992), Parent et al. (1994), and Khiari et al. (2001). CND norms can also be used for diagnostic purposes:

$$I_{\mathrm{N}} = \frac{V_{\mathrm{N}} - V_{\mathrm{N}}^*}{\mathrm{S.D.}_{\mathrm{N}}^*},\ I_{\mathrm{P}} = \frac{V_{\mathrm{P}} - V_{\mathrm{P}}^*}{\mathrm{S.D.}_{\mathrm{P}}^*},$$

$$I_{\mathrm{K}} = \frac{V_{\mathrm{K}} - V_{\mathrm{K}}^*}{\mathrm{S.D.}_{\mathrm{K}}^*}, \ldots, I_{R_d} = \frac{V_{R_d} - V_{R_d}^*}{\mathrm{S.D.}_{R_d}^*}, \tag{10}$$

where $I_{\mathrm{N}}, \ldots, I_{R_d}$ are the CND indices.

Independence among compositional data is ascertained by row-centered log ratio transformation (Aitchison, 1986). 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 hypershpere ($d+1>3$) in a $d+1$ dimensional space. The CND nutrient imbalance index of a diagnosed specimen is its CND r^2 and is computed by:

$$r^2 = I_{\mathrm{N}}^2 + I_{\mathrm{P}}^2 + I_{\mathrm{K}}^2 + \cdots + I_{R_d}^2. \tag{11}$$

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-square 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-square 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-square cumulative distribution function. As defined by Eqs. (10) and (11), the closer to zero that CND indices and thus the CND r^2 or chi-square values are, the higher probability to obtain a high yield. Theoretically, at the critical chi-square value of zero where the ideal nutrient balance is reached, 100% of the population would be expected to produce low target yields when a high critical chi-square value is set.

2.2. Experimental data

This study is based on data acquired from a survey conducted on the experimental field 'Centro de Propagación Vegetativa' of the 'Centro de Investigaciones Biológicas del Noroeste, SC' (CIBNOR, SC), located in El Carrizal, BCS, Mexico. This area of the Baja California Peninsula is situated between 19° and 31°N, where most dry regions in the world are located, and is one of the driest (Aguilera and Martínez, 1996). Its climate is classified as Bw (h′) hw (e) (García, 1981). Yearly mean temperature is between 22 and 23 °C, and the annual mean precipitation is between 100 and

Table 1
Soil properties at the experimental site

Property	Mean ± S.D.	Interpretation of nutrient availability
Total N	4.88 ± 0.93 (mg N kg^{-1})	–
NO_3-N	2.71 ± 0.44 (mg NO_3 kg^{-1})	Low
NH_4-N	0.47 ± 0.62 (mg NH_4 kg^{-1})	Low
Extractable P	23.74 ± 12.45 (mg P kg^{-1})	High
Exchangeable Ca	25.3 ± 6.93 (mg Ca kg^{-1})	Low
Exchangeable Mg	10.38 ± 3.78 (mg Ca kg^{-1})	Low
Soluble K	54.04 ± 13.8 (mg K kg^{-1})	Low
$CaCO_3$	18.5 ± 4.5 (mg $CaCO_3$ kg^{-1})	–
pH	6.85 ± 0.25	Neutral
Electrical conductivity	1.02 ± 0.20 (dS m^{-1})	Low

250 mm, while the yearly mean evaporation varies from 1758 to 2472 mm (INEGI, 1997).

The cultivar was sown on February 2003. Experimental plot size was 1 ha. Row distance was 180 cm and plant distance was 80 cm, such that the population density was 6936 plants ha^{-1}. Plants were irrigated by drip irrigation at an average rate of 3 mm day^{-1}. Herbicide, fungicide, and insecticide treatments were applied according to standard cultural practices.

The soil was a *loamy coarse sand* (Bouyocus procedure, particle counter) containing 0.4% organic matter (Walkley–Black procedure, muffle furnace with correction for $CaCO_3$). Before sowing, 10 soil samples from the 0 to 30 cm layer were analysed for total N using the Kjeldahl approach (Jackson, 1958; Bremner and Mulvaney, 1982), and P content by the Olsen technique (Olsen and Sommers, 1982) (the P content was highly variable; coefficient of variation = 52%). Extractable Ca and Mg were measured by the volumetric method (Cheng and Bray, 1951), extractable K was measured by Peech method (Knudsen et al., 1982), pH at a solution ratio 1:2 (deionised-water:soil), electrical conductivity using a conductivity meter (Rhoades, 1982), and $CaCO_3$ content using the titration with acid method (USSL, 1954). All values of soil properties are shown in Table 1. Local soil is classified by the FAO system as *Haplic Yermosol* (INEGI, 1997). Fujiyama (2001) has reported that a soil with these characteristics could also be classified as a desert calcareous soil.

A total of 64 foliar composite samples were collected from randomly chosen healthy plants throughout the experimental plot at eight months after sowing. Each sample corresponds to three mature leaves from sampled plant. Each sample was cleaned with distilled water, dried to constant mass and analysed for total N, P, K, Ca, and Mg. Total N content was determined by the Kjeldahl method. K, Ca, and Mg were estimated by using an atomic absorption spectrophotometer (Shimadzu AA-660, Shimadzu, Kyoto, Japan) after digestion with H_2SO_4, HNO_3, and $HClO_4$ mixture. P was estimated colorimetrically by the phospho-molybdate blue complex method.

The plants used for foliar samples were harvested; then each observation in the nutrient dataset was matched with yield from the same plant. Harvested fresh matter was considered as yield. Thus, used dataset included yield and N, P, K, Ca, and Mg tissue concentrations. Compositional nutrient diagnosis norms were calculated using Microsoft Excel 2000 Software (Microsoft Corp., 2000), and principal component analyses (PCA) were performed using factor analysis module of the Statistica package version 6 (StatSoft Inc., 2004). Additionally, it deserves to be mentioned that in order to identify nutrient expressions related to yield, and to know the important nutrient interactions level of responsibility on the discrimination between low- and high-yielding subpopulations, a correlation matrix, and *t*- and *F*-tests were carried out.

3. Results and discussion

3.1. Descriptive statistics of yield

The descriptive statistics of the yield were as follows: mean = 132.97 t ha^{-1}, minimum = 58.96 t ha^{-1}, maximum = 246.28 t ha^{-1}, and standard deviation = 40.96 t ha^{-1}.

3.2. The compositional nutrient diagnosis norms for simplex S^5

The S^5, i.e., six-dimensional $(d+1)$ *A. vera* simplex comprised the five nutrients N, P, K, Ca, and Mg and the filling value R (Khiari et al., 2001; Magallanes-Quintanar et al., 2004). Nutrient concentrations were transformed into CND row-centered log ratios V_N, V_P, V_K, V_{Ca}, and V_{R_d} through the Eqs. (1)–(4). Eq. (7) was used to calculate the cumulative variance ratio functions $[F_i^C(V_X)]$ values.

The cutoff yield between the low- and high-yield subpopulations was determined after examining the five 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 $F_i^C(V_R)]$ related to yield (Fig. 1). All six relationships showed a cubic pattern (Fig. 2) with inflection points at $-b/3a$ (Eq. (9)). Yield cutoff values were 126.67 t ha^{-1} for $F_i^C(V_N)$, 98.33 t ha^{-1} for $F_i^C(V_P)$, 125.56 t ha^{-1} for $F_i^C(V_K)$, 86.67 t ha^{-1} for $F_i^C(V_{Ca})$, 136.67 t ha^{-1} for $F_i^C(V_{Mg})$, and 323.33 t ha^{-1} for $F_i^C(V_R)$

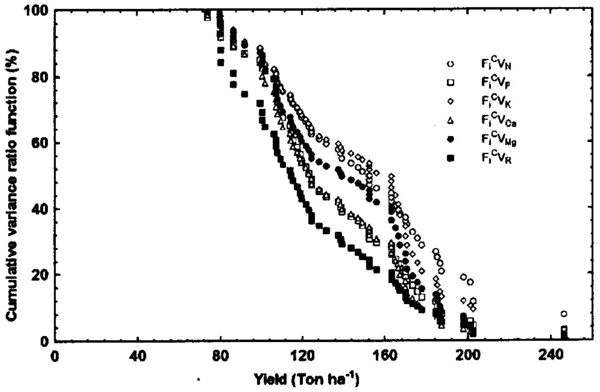

Fig. 1. Relationship between *Aloe vera* yield and cumulative variance ratio functions in S^5 for computing yield cutoff between high- and low-yielding subpopulations at inflection point in the cubic pattern.

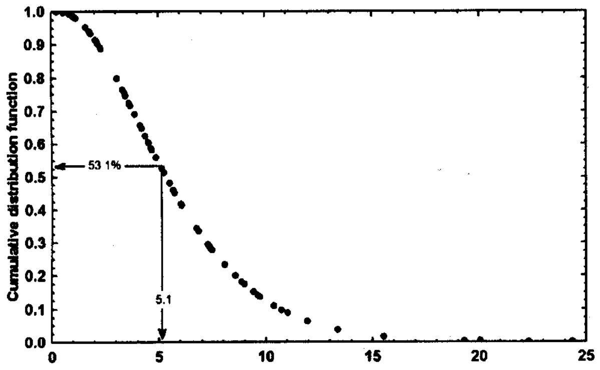

Fig. 2. The chi-square cumulative distribution function with 6 d.f. for obtaining theoretical threshold compositional nutrient diagnosis (CND) r^2 value (5.1) in S^5 for yield cutoff at 53.1% of low-yielding subpopulation.

(Table 2). The theory of the CND approach recommends that the highest yield cutoff value (highest discrimination power) among $d+1$ nutrient computations must be retained to calculate the proportion of the low-yield subpopulation below yield cutoff used as the critical value for the chi-square cumulative distribution function. In the present case, it is noted that the highest value (323.33 t ha^{-1}) is out of the explored yield range, so it was not considered as target yield. However, 136.67 t ha^{-1} was used to define the high-yielding subpopulation. This result implies that 46.9% of the population (30 observations from 64) is considered as the high-yielding subpopulation. Parent et al. (1994) have retained around 30% of the population in previous experiences.

The preliminary CND norms, as means and standard deviations (V_X^* and S.D.$_X^*$, respectively) of the CND row-centered log ratios for the high-yield subpopulation (Table 3), were used to estimate nutrient indices I_N, I_P, I_K, I_{Ca}, I_{Mg}, and I_{R_d} and CND r^2 values using the Eqs. (10) and (11). The CND r^2 values were distributed like chi-square values ($R^2 > 0.999$; $p < 0.001$) (Fig. 2). There, 53.1% of the observations were below the yield cutoff of 136.67 t ha^{-1}, while the corresponding chi-square value with 6 degrees of freedom was 5.1 (Fig. 2); then, this value must be considered when validating the preliminary CND norms because the independent dataset ought to be characterized by a similar value. By taking into account that more high-yielding specimens must be added to the database, as pointed out previously, the chi-square value of 5.1 (Fig. 2) could change because the high-yielding subpopulation could provide more weight for defining yield cutoff than the low-yielding subpopulation according to the theory of the CND approach.

The optimum ranges for nutrient concentrations associated to the preliminary CND norms are presented in Table 3. The increasing order of mineral nutrients as accumulated in *A. vera* leaves is as follows: P > N > Mg > K > Ca. The importance of N has been evaluated on theoretical grounds because it may be pointed out that CAM plants might need less N than C_3 plants, and thus having higher nitrogen use efficiency (Raven and Spicer, 1996). In our case, low N accumulation in *A. vera* leaves could also be due to the low availability of NO_3-N and NH_4-N in the soil (Table 1). However, really there is no clear evidence supporting particularly high nitrogen use efficiency in CAM plants (Lüttge, 2004) including *A. vera*. What is remarkable is that although P was highly available in the soil (Table 1), *A. vera* plants did not tend to accumulate P in their leaves in higher quantities (Table 3) suggesting a high phosphorus use efficiency, which deserves be studied. In general, our results agree with that reported previously in the context that in the chlorenchyma of cultivated cacti and agaves, levels of Ca and Mg tended to be higher than in most other agronomic plants (Lüttge, 2004), because both mineral nutrients plus K had low availability in the soil (Table 3); thus *A. vera* can be considered as calcitrophic specie.

A large body of literature has shown linkages between foliar chemistry and various ecosystem processes (photosyn-

Table 2
Yield of *Aloe vera* at inflection points of cumulative variance functions for row-centered log ratios ($n = 61$) in the survey population ($n = 64$)

Nutrient	$F_i^C(V_X) = aY^3 + bY^2 + cY + d$	R^2	Yield at inflection point ($-b/3a$) (t ha^{-1})
N	$y = 0.00002x^3 - 0.0076x^2 + 0.3067x + 110.12$	0.993	126.67
P	$y = 0.00002x^3 - 0.00591x^2 - 0.3516x + 151.57$	0.986	98.33
K	$y = 0.00003x^3 - 0.0113x^2 + 0.8618x + 86.643$	0.975	125.56
Ca	$y = 0.00002x^3 - 0.0052x^2 - 0.4665x + 156.93$	0.989	86.67
Mg	$y = 0.00002x^3 - 0.0082x^2 + 0.2083x + 120.87$	0.981	136.67
R	$y = -0.00001x^3 + 0.0097x^2 - 2.7383x + 253.55$	0.992	323.33

Table 3
The compositional nutrient diagnosis (CND) norms for $d=5$ nutrients, and optimum ranges (mean ± standard deviation, S.D.) of nutrients for *Aloe vera* production with a yield cutoff value of reference of 136.67 t ha^{-1}

Row-centered log ratio	Mean	S.D.	Nutrient	Mean (g kg^{-1})	S.D. (g kg^{-1})
V_N^*	−1.033	0.105	N	8.13	1.34
V_P^*	−2.617	0.142	P	1.68	0.34
V_K^*	−0.041	0.201	K	22.39	6.03
V_{Ca}^*	0.692	0.168	Ca	45.42	5.55
V_{Mg}^*	−0.700	0.128	Mg	11.33	1.69
$V_{R_5}^*$	3.699	0.104	*R*	911.03	74.91
$\sum V_X$	0	–	–	–	–

thesis, decomposition, etc.) and recent advances with hyperspectral resolution remote sensing make detection across large, heterogeneous landscapes possible (Aber and Melillo, 2001). In this sense, limitation by a nutrient is shown if the rate of an ecosystem process is increased by addition of that nutrient, and strictly speaking it can only be determined experimentally (Tanner et al., 1998). Furthermore, while overall ecosystem processes may be nutrient limited, not all species in the ecosystem need be limited; indeed even within a species some individuals could be limited and others not, due, for example, to different crown exposure. Finally, the observation that a nutrient is limiting does not mean that only that nutrient limits the ecosystem—simultaneous limitation by multiple resources is the rule, especially with different types of resources, e.g. photosynthetically active radiation, water, depletable resources like N or P, and non-depletable resources like CO_2 (Bloom et al., 1980; Field et al., 1992).

3.3. Nutrient interactions

A principal component analysis (PCA) was conducted on CND indexes considering three cases: the whole dataset (64 observations), the high-yielding subpopulation (30 observations), and the low-yielding subpopulation (34 observations). Significant factor loadings were obtained as suggested by Ovalles and Collins (1988) and Gutiérrez-Acosta et al. (2002) after varimax rotation. In the first case (whole population), the first two PCs explained 77.36% of total variance (Table 4). On the other hand, the two first PCs in the cases of high- and low-yielding subpopulations explain 75.98 and 80.01%, respectively (Table 4). It is appreciated in all the three cases that the structures of the two PCs are almost similar, that is, they present almost the same important loadings in each of the two first PCs.

For the whole population and the high-yielding subpopulation cases, the first PC presents two positive interactions: I_P–I_K and I_{Ca}–I_{Mg}, and four negative correlations: I_P–I_{Ca}, I_P–I_{Mg}, I_K–I_{Ca}, and I_K–I_{Mg}; suggesting the following significant positive interactions: P–K and Ca–Mg, which means that *A. vera* plants tend to accumulate both nutrients (in each couple) in its leaves; and the following negative interactions: P–Ca, P–Mg, K–Ca, and K–Mg, meaning the tendency of *A. vera* plants to accumulate one and does not accumulate the other nutrient in each interaction (Table 4). Also, in both cases, the second PC was correlated positively with I_N and negatively with I_R, indicating a negative interaction between N and the filling nutrient *R* (Table 4). For the low-yielding subpopulations, the structure of the PC1 is defined by I_P, I_K, I_{Ca}, and I_{Mg} as were the results for the whole population and the high-yielding subpopulation; however, the structure of the PC2 is only defined by an important positive loading associated to I_N; it is notable that I_R is not as important in this PC2 as in the high-yielding subpopulation.

Therefore, under the consideration of the structure of PC2 in all three cases, there is the possibility that N or *R* could be depressing yield when taking into account the whole dataset

Table 4
Loadings or correlations between the first two principal components and the CND indexes for the whole dataset (64 observations), the high-yielding subpopulation (27 observations), and the low-yielding subpopulation (37 observations), extracted from varimax normalized matrix

CND indexes	PC1[a]	PC2[a]	PC1[b]	PC2[b]	PC1[c]	PC2[c]
I_N	0.097	**0.926**	0.112	**0.857**	0.078	**−0.955**
I_P	**−0.849**	0.041	**−0.836**	0.062	**−0.858**	−0.101
I_K	**−0.886**	0.091	**−0.900**	0.191	**−0.883**	0.090
I_{Ca}	**0.894**	−0.227	**0.858**	−0.397	**0.912**	0.120
I_{Mg}	**0.831**	−0.024	**0.803**	0.112	**0.874**	0.066
I_R	0.491	**−0.689**	0.388	**−0.749**	0.618	0.592
Explained variance	3.247	1.395	3.052	1.507	3.500	1.300
Proportion of total	0.541	0.232	0.509	0.251	0.583	0.217

Values in boldface are the dominant in the eigenvector loadings by setting the level of significance at approximately 0.65.

[a] Values from whole dataset.

[b] Values from high-yielding subpopulation.

[c] Values from low-yielding subpopulation.

Table 5
Correlation matrix between yield and CND indexes for the whole dataset ($n = 64$)

	Yield	I_N	I_P	I_K	I_{Ca}	I_{Mg}
I_N	**0.29**					
I_P	−0.08	−0.08				
I_K	0.13	−0.02	**0.53**			
I_{Ca}	0.05	−0.16	**−0.80**	**−0.78**		
I_{Mg}	−0.07	−0.09	**−0.59**	**−0.77**	**0.65**	
I_R	**−0.36**	**−0.40**	**−0.51**	**−0.52**	**0.51**	**0.25**

Pearson correlations in boldface are significant at $p < 0.05$.

or at least the high-yielding subpopulation. Furthermore, results allowed us to hypothesise that the identified negative interaction N–R could be a significant discriminator, among others, between the high- and low-yielding subpopulations. Additionally, with the aim of elucidating such hypotheses, two statistical analyses were performed. The first one consisted of carrying out a correlation matrix to test if yield depends strongly on CND indexes; and the late involved identified important nutrient interactions in t- and F-tests in order to estimate levels of significance in the discrimination between high- and low-yielding subpopulations.

Pearson correlations between CND indexes (I_X) and yield are provided (Table 5). There is remarkable that *A. vera* yield depends on N positively and on R negatively. This result means that N increase and R depress yield. In addition, this matrix also allowed us to identify the following significant ($p < 0.05$) interactions: positively for P–K, Ca–Mg, Ca–R, and Mg–R; negatively for N–R, P–Ca, P–Mg, P–R, K–Ca, K–Mg, and K–R. By this way, the Ca–R, Mg–R, P–R and K–R interactions were the interactions no identified by the principal component analyses previously described, suggesting the important role of the filling nutrient R in *A. vera*.

Some of the elucidated interactions are not fully understood under physiological basis or little information regarding them has been reported for *A. vera*. The elucidated P–K synergism does not have a robust physiological explanation; really, little progress has been reached on P–K interactions. However, Reneau et al. (1983), cited by Sumner and Farina (1986), have demonstrated that P–K interaction is important in forage sorghum production. Also, Sumner and Farina (1986) pointed out that the balance between K and P is important. Thus, we can conclude that this interaction is an important synergism when plants present the following concentration ranges: 1–3.1 g P kg^{-1} and 12.45–40.9 g K kg^{-1} under dry matter basis in leaves of *A. vera*.

The identified positive Ca–Mg interaction agrees with the performance of nutrient interactions in *Capsicum annuum* (García-Hernández et al., 2004) and *Vigna unguiculata* (García-Hernández et al., 2005). In both of these cases, plants were growing under similar soil and environmental conditions as *A. vera* did, additionally, readers must keep in mind that is a calcitrophic specie as pointed out previously. Raghupathi et al. (2002) found also a positive Ca–Mg interaction in leaves of banana plants growing on Aquic Haplustalfs. However, commonly, Ca–Mg interaction is found as negative interaction considering that commonly Ca^{2+} is strongly competitive with Mg^{2+} in substrates and often result in increased leaf-Ca along with a marked reduction in leaf Mg in many crops (Ruiz et al., 1997; Grattan and Grieve, 1999; Appenroth et al., 1999). Moreover, it is well known that Mg^{2+} is more soluble in the soil solution and hence more easily lost. As a concluding remark on this interaction, we are able to point out that *A. vera* probably is a specie tending to accumulate Ca and Mg when plants present the following concentration ranges: 29.7–59.9 g Ca kg^{-1} and 7.6–13.9 g Mg kg^{-1} under dry matter basis in their leaves.

The P–Ca antagonism has been reported by Parent et al. (1994), among other authors. Despite the high P availability in the soil (Table 1), *A. vera* plants did tend to accumulate less P as increasing Ca leaves concentration. This could be due to the calcareous nature of the soil, resulting in exchangeable Ca^{2+} forming insoluble P precipitates (Tunesi et al., 1999) and thus, reducing the P availability to the plants. Moreover, this suggestion could be reinforced by the idea pointed out before, concerning to *A. vera* plants show a high phosphorus use efficiency. Certainly, this is what happens when plants present the following concentration ranges: 29.7–59.9 g Ca kg^{-1} and 1–3.1 g P kg^{-1} under dry matter basis in their leaves.

The negative P–Mg relationship could be related to plant metabolism and to dilution–accumulation process over time (Marschner, 1986; Walworth and Sumner, 1987). Additionally, it deserves be highlighted that Mg concentration is much higher than that for P in *A. vera* (Table 3). This result might be reinforced by the trend of *A. vera* plants to accumulate larger amounts of Mg than that reported for most of the common crops as pointed out ut supra. However, it has been reported the fact that Mg is an activator for almost all reactions involving phosphate transfer within the plant (Sumner and Farina, 1986). Unfortunately, little relevant work has been conducted under field conditions regarding quantitative assessments of the importance of this interaction. As a remarkable result of this research work, it is clear this antagonism probably occurs when plants present the following concentration ranges: 1–3.1 g P kg^{-1} and 7.6–13.9 g Mg kg^{-1} under dry matter basis in leaves of *A. vera*.

Another important interaction was the K–Ca antagonism (Table 4). Marschner (1986) has already reported this antagonism, and Walworth and Sumner (1987) as a contrast related to K dilution and Ca accumulation over time. Thus, this result agrees with the soil calcareous nature and the low K solubility in the substrate (Table 1). By this way, there is the possibility that K dilution does not occur when plants accumulate enough K kg^{-1} under dry matter basis in leaves of *A. vera* and present lower Ca leaf concentration without ability to promote K dilution.

The K–Mg negative relationship has even a smaller physiological explanation, but was reported earlier for banana (Raghupathi et al., 2002).

As it is understood, R interacted significantly with N, Ca, and Mg. So, an independent t-tests for all six (five plus R)

Table 6
Basic statistics of the high- and low-yielding subpopulations, and t- and F-tests for the identified important nutrient interactions

Nutrient ratio	High-yielding subpopulation ($n = 30$)		Low-yielding subpopulation ($n = 34$)		t-Value	p-Value	F-value	p-Value
	Mean	S.D.	Mean	S. D.				
P/K	0.077	0.013	0.081	0.015	−1.230	0.223	1.518	0.257
P/Ca	0.038	0.013	0.040	0.015	−0.533	0.596	1.325	0.445
P/Mg	0.151	0.038	0.154	0.049	−0.230	0.819	1.627	0.186
K/Ca	0.512	0.199	0.498	0.166	0.309	0.758	1.441	0.309
K/Mg	2.026	0.659	1.922	0.582	0.667	0.507	1.283	0.487
Ca/Mg	4.063	0.604	3.919	0.450	1.087	0.281	1.800	0.104
N/R	0.009	0.002	0.008	0.001	**2.458**	0.017	1.282	0.488
P/R	0.002	0.0004	0.002	0.0005	0.431	0.668	1.766	0.123
K/R	0.025	0.007	0.022	0.007	1.319	0.192	1.143	0.707
Ca/R	0.050	0.007	0.046	0.006	**2.204**	0.031	1.150	0.693
Mg/R	0.012	0.002	0.012	0.001	1.387	0.170	**2.064**	0.046

t- and F-values in boldface are significant at $p < 0.05$.

nutrients was carried out. Results indicated that N ($t = 2.39$, $p < 0.02$), Ca ($t = 2.08$, $p < 0.04$), and R ($t = -2.94$, $p < 0.004$) are important discriminators between the high- and low-yielding subpopulations as well as N/R, Ca/R, and Mg/R ratios (Table 6) suggesting the importance of the balance between the filling nutrient R with N, Ca, and Mg in *A. vera*.

Data in Table 3 allows anyone to infer that *A. vera* plants accumulate five and three times more Ca and K, respectively, than N. However, such situation does not indicate N is not an important nutrient in *A. vera* as it is demonstrated through its roles in the yield dependence on it (Table 5) and in the negative interaction with the filling value R to discriminate between high-and low-yielding subpopulations (Table 6). In this context, we conclude that ecophysiologically it is important to consider that N leaf concentration in relation to the action of other factors, especially nutrients, light and water.

In summary, research on these topics and other nutrient interactions occurring in *A. vera* must be carried out in the near future in order to understand their roles as limiting factors of *A. vera* plant growth under field conditions. The interpretation of interactions identified by diagnostic techniques, as the multivariate CND approach, could help in overcoming some of the drawbacks of the classical approaches because they have the ability to handle interactions between nutritional factors in terms of scale invariant proportions.

4. Conclusions

The preliminary compositional nutrient diagnosis (CND) norms expressed as row-centered log ratios (means ± standard deviation) for $d = 5$ nutrients in a high-yielding subpopulation producing more than 136.67 t ha^{-1} of *A. vera* growing on calcareous soils in arid environment are: $V_{\mathrm{N}}^* = -1.033 \pm 0.105$, $V_{\mathrm{P}}^* = -2.617 \pm 0.142$, $V_{\mathrm{K}}^* = -0.041 \pm 0.201$, $V_{\mathrm{Ca}}^* = 0.692 \pm 0.168$, $V_{\mathrm{Mg}}^* = -0.7 \pm 0.128$, and $V_{R_5}^* = 3.699 \pm 0.104$.

These compositional nutrient diagnosis norms for fresh matter yields higher than 136.67 t ha^{-1} are associated with the following foliar ranges: $8.13 \pm 0.1.134$ g N kg^{-1}, 1.68 ± 0.34 g P kg^{-1}, 22.39 ± 6.03 g K kg^{-1}, 45.42 ± 5.55 g Ca kg^{-1}, and 11.33 ± 1.69 g Mg kg^{-1}.

The positive interactions P–K and Ca–Mg, and the negative interactions P–Ca, P–Mg, K–Ca, K–Mg, and N–R were identified. By using Pearson correlations, the same interactions were identified plus the following: negatively P–R and K–R, and positively Ca–R and Mg–R.

Estimated t- and F-values and their corresponding probability levels indicated that N/R antagonism, Ca/R and Mg/R synergisms, and nutrients N, Ca, and R were the factors discriminating high- from low-yielding subpopulations.

Acknowledgements

Research was supported by the Japanese International Cooperation Agency, Tottori University, Fundación PRODUCE BCS, and CIBNOR Project ZA1.2.

References

Aber, J.D., Melillo, J.M., 2001. Terrestrial Ecosystems, second ed. Academic Press, 556 pp.

Aguilera, C.M., Martínez, R., 1996. Relaciones Agua, Suelo, Planta, Atmósfera. Universidad Autónoma Chapingo, México.

Aitchison, J., 1986. Statistical Analysis of Compositional Data. Chapman and Hall, New York.

Adusumilli, P.S., Ben-Porat, L., Pereira, M., Roesler, D., Leitman, I.M., 2004. The prevalence and predictors of herbal medicine use in surgical patients. J. Am. Coll. Surgeons 198, 583–590.

Alegre, J., López-Vela, D., Eymar, E., Alonso-Blázquez, N., Yébenes, L., 2003. Evaluating bearberry nitrogen nutrition using hydropnic cultures: Establishing preliminary DRIS norms. J. Plant Nutr. 26, 525–542.

Appenroth, K.J., Gbrys, H., Scheuerlein, R.W., 1999. Ion antagonism in phytochrome-mediated calcium-dependent germination of turions of *Spirodela polyrhiza* (L.). Schleiden Planta 208, 583–587.

Bates, T.E., 1971. Factors affecting critical nutrient concentrations in plant and their evaluation, a review. Soil Sci. 112, 116–130.

Bloom, A.J., Mooney, H.A., Bjorkman, O., Berry, J., 1980. Materials and methods for carbon dioxide and water exchange analysis. Plant Cell Environ. 3, 371–376.

Borland, A.M., Taybi, T., 2004. Synchronization of metabolic processes in plants with Crassulacean acid metabolism. J. Exp. Bot. 55 (400), 1255–1265.

Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2. Chemical and Microbiological Properties. ASA Monograph 9. Madison, WI, pp. 595–624.

Cheng, K.L., Bray, R.H., 1951. Determination of calcium and magnesium in soil and plant material. Soil Sci. 72, 449–458.

Escano, C.R., Jones, C.A., Uehara, G., 1981. Nutrient diagnosis in corn grown in hydric dystrandepts. II. Comparison of two systems of tissue diagnosis. Soil Sci. Am. J. 45, 1140–1144.

Field, C.B., Chapin III, F.S., Matson, P.A., Mooney, H.A., 1992. Responses of terrestrial ecosystms to the changing atmosphere. Annu. Rev. Ecol. Syst. 3, 371–376.

Fujiyama, H., 2001. Salinity problems in arid lands agriculture. In: Textbook for Training Course in Irrigation Water Resources in Arid & Semi-arid Region and E.I.A. for Sustainable Development. Tottori University-JICA, Japan, pp. 210–225.

García, E., 1981. Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía, Universidad Nacional Autónoma de México, México.

García-Hernández, J.L., Valdez-Cepeda, R.D., Murillo-Amador, B., Nieto-Garibay, A., Beltrán-Morales, L.F., Magallanes-Quintanar, R., Troyo-Diéguez, E., 2004. Compositional nutrient diagnosis and main nutrient interactions in yellow pepper grown on desert calcareous soils. J. Plant Nutr. Soil Sci. 167, 509–515.

García-Hernández, J.L., Valdez-Cepeda, R.D., Ávila-Serrano, N.Y., Murillo-Amador, B., Nieto-Garibay, A., Magallanes-Quintanar, R., Larrinaga-Mayoral, J., Troyo-Diéguez, E., 2005. Preliminary compositional nutrient diagnosis norms for cowpea (*Vigna ungiculata* (L.) Walp.) grown on desert calcareous soil. Plant Soil 271, 297–307.

Grattan, S.R., Grieve, C.M., 1999. Salinity-mineral nutrient relations in horticultural crops. Sci. Hort. 78, 127–157.

Gutiérrez-Acosta, F., Valdez-Cepeda, R.D., Blanco-Macías, F., 2002. Multivariate analysis of cactus pear (*Opuntia* spp.) fruits from a germplasm collection. Acta Hort. 581, 111–118.

INEGI (Instituto Nacional de Estadística, Geografía e Informática), 1997. Síntesis geográfica del estado de Baja California Sur. INEGI, Aguascalientes, México.

Jackson, M.L., 1958. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ, USA.

Khiari, L., Parent, L.E., Tremblay, N., 2001. Selecting the high-yield subpopulation for diagnosing nutrient imbalance in crops. Agron. J. 93, 802–808.

Knudsen, D., Peterson, G.A., Prat, P.F., 1982. Lithium, sodium and potassium. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2. Chemical and Microbiological Properties. ASA Monograph 9. Madison, WI, pp. 403–430.

Lüttge, U., 2004. Ecophysiology of crassulacean acid metabolism (CAM). Ann. Bot. 93, 629–652.

Magallanes-Quintanar, R., Valdez-Cepeda, R.D., Blanco-Macías, F., Márquez-Madrid, M., Ruiz-Garduño, R.R., Pérez-Veyna, O., García-Hernández, J.L., Murillo-Amador, B., López-Martínez, J.D., Martínez-Rubín de Celis, E., 2004. Compositional nutrient diagnosis in nopal (*Opuntia ficus-indica*). J. Prof. Assoc. Cactus Develop. (6), 78–89.

Marschner, H., 1986. Mineral Nutrition of Higher Plants. Academic Press, London.

Mattos Jr., D., Quaggio, J.A., Cantarella, H., 2003. Nutrient content of biomass components of Hamlin sweet orange trees. Sci. Agric. 60, 155–160.

Microsoft Corp., 2000. Microsoft Excel 2000 (Computer Program Manual). Troy, NY, USA.

Moody, J.O., Adebiyi, O.A., Adeniyi, B.A., 2004. Do *Aloe vera* and *Ageratum conyzoides* enhance the anti-microbial activity of traditional medicinal soft soaps (Osedudu)? J. Ethnopharmacol. 92, 57–60.

Navvabzdeh, M., Malakouti, M.J., 1993. Development of DRIS norms for potato in the calcareous soils of Iran. J. Plant Nutr. 16, 1409–1416.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2. Chemical and Microbiological Properties. ASA Monograph 9. Madison, WI, pp. 403–430.

Orafidiya, L.O., Agbani, E.O., Oyedele, A.O., Babalola, O.O., Onayemi, O., Aiyedun, F.F., 2004. The effect of *Aloe vera* gel on the anti-acne properties of the essential oil of *Ocimum gratissimum* Linn leaf—a preliminary clinical investigation. Int. J. Aromather. 14, 15–21.

Ovalles, F.A., Collins, M.E., 1988. Variability of northwest Florida soils by principal component analysis. Soil Sci. Soc. Am. J. 52, 1430–1935.

Parent, L.E., Dafir, M., 1992. A theoretical concept of compositional nutrient diagnosis. J. Am. Soc. Hort. Sci. 117, 239–242.

Parent, L.E., Cambouris, A.N., Muhawenimana, A., 1994. Multivariate diagnosis of nutrient imbalance in potato crops. Soil Sci. Soc. Am. J. 58, 1432–1438.

Parihar, M.S., Chaudhary, M., Shetty, R., Hemnani, T., 2004. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of *Withania somnifera* and *Aloe vera*. J. Clin. Neurosci. 11, 397–402.

Raven, J.A., Spicer, R.A., 1996. The evolution of crassulacean acid metabolism. In: Winter, K., Smith, J.A.C. (Eds.), Crassulacean Acid Metabolism. Biochemistry, Ecophysiology and Evolution. Ecological Studies, vol. 114. Springer-Verlag, Berlín, Heidelberg, New York, pp. 360–385.

Raghupathi, H.B., Reddy, B.M.C., Srinivas, K., 2002. Multivariate diagnosis of nutrient imbalance in banana. Commun. Soil. Sci. Plant Anal. 33 (13&14), 2131–2143.

Rhoades, J.D., 1982. Soluble salts. In: Page, A.L. (Ed.), Methods of Soil Analysis: Part 2. Chemical and Microbiological Properties. ASA Monograph 9, Madison, WI, pp. 167–169.

Ross, S.M., 1987. Introduction to Probability and Statistics for Engineers and Scientists. John Wiley & Sons, New York, USA.

Ruiz, D., Martínez, B., Cerdá, A., 1997. Citrus response to salinity, growth and nutrient uptake. Tree Physiol. 17, 141–150.

StatSoft Inc., 2004. Statistica for Windows (Computer Program Manual). StatSoft, Inc., Tulsa, OK, USA.

Strahler, A.N., Strahler, A.H., 1984. Elements of Physical Geography. Wiley and Sons, Londres.

Sumner, M.E., Farina, M.P.W., 1986. Phosphorus interactions with other nutrients and lime in field cropping systems. Adv. Soil Sci. 5, 2101–2236.

Tanner, E.V.J., Vitousek, P.M., Cuevas, E., 1998. Experimental investigation of nutrient limitation of forest growth on wet tropical mountains. Ecology 79 (1), 10–22.

Tunesi, S., Poggi, V., Gessa, C., 1999. Phosphate adsorption and precipitation in calcareous soils, the role of calcium ions in solution and carbonate minerals. Nutr. Cycl. Agroecosys. 53, 219–227.

USSL (U.S. Salinity Laboratory), 1954. Carbonate and bicarbonate by titration with acid. In: Richards, L.A. (Ed.), Diagnosis and Improvement of Saline and Alkali Soils. USDA Agricultural Handbook, vol. 60. U.S. Government Printing Office, Washington, DC, pp. 98–108.

Vazquez, B., Avila, G., Segura, D., Escalante, B., 1996. Anti-inflammatory activity of extracts from *Aloe vera* gel. J. Ethnopharmacol. 55, 69–75.

Walworth, J.L., Sumner, M.E., 1987. The diagnosis and recommendation integrated system (DRIS). Adv. Soil Sci. 6, 149–188.

Walworth, J.L., Woodard, H.J., Sumner, M.E., 1988. Generation of corn tissue norms from a small, high-yield database. Commun. Soil Sci. Plant Anal. 19, 563–577.

Ware, G.O., Ohki, K., Moon, L.C., 1982. The Mitscherlich plant growth model for determining critical nutrient deficiency levels. Agron. J. 74, 88–91.

Yusuf, S., Abdulkarim, A., Mshelia, D., 2004. The effect of *Aloe vera* A. Berger (Liliaceae) on gastric acid secretion and acute gastric mucosal injury in rats. J. Ethnopharmacol. 93, 33–37.