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Establishment of nutrients optimal range for nutritional diagnosis of mandarins based on DRIS and CND methods

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ABSTRACT

The aim of this work was to establish for the first time nutrient optimal range (NOR) for mandarins in Tunisia using two methods: Diagnosis and Recommendation Integrated System (DRIS) and Compositional Nutrient Diagnosis (CND). The study was performed using data from 120 mandarin's commercial orchards, in several locations in Tunisia. Yield data and leaf nutrient content from 2011 to 2014 cropping season were compiled in a database. Leaf samples consist of spring flush leaves (7–8 months old) collected from nonfruiting terminals. The two methods were effective to diagnose the plant nutritional status based on the effectiveness of the chi-square-tested method. The two methods showed the nutrient imbalances of the studied commercial orchards. Phosphorus (P) and iron (Fe) showed higher frequencies as the most required nutrients by the plant. The found norms were then compared to the norms used in other countries. The new norms for mandarins are different because some norms in the literature do not take into account the different species of citrus, and they were adopted in different pedoclimatic conditions. So, adopting the new leaf nutrient norms is key for ensuring productivity in mandarin citrus orchards in Tunisia.

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Introduction

Citrus production is considered the most important fruit in the world. Citrus in Tunisia is a strategic production that occupies an important place in the socioeconomic life of the country. The fertigation and phytosanitary control of citrus, two important technical aspects not well controlled by the growers are responsible for the low productivity of this sector (Kalai et al. 2010). Nutrient deficiency or excess will cause citrus trees to grow poorly and produce sub-optimal yield and fruit quality. Diagnosis of potential nutritional problems should be a routine citrus-growing practice. Quantifying nutrients in soils and trees eliminates guesswork when adjusting a fertilizer program (Obreza and Morgan 2008). The leaf analysis norms are used to estimate citrus tree nutrition. The assessment of nutritional status of citrus orchards, based on the interpretation of leaf analysis, has been widely used in other regions and discussed in order to enable more precise interventions through management and fertilization practices (Menino 2012). A variety of interpretation tools have shown their usefulness in leaf analysis of citrus. These are: critical nutrient concentration (Srivastava et al. 1999; Terblanche and Du Plessis 1992), nutrient concentration range (Parent and Dafir 1992), nutrient balance using factorial method (Cantarella et al. 1992), Kenworthy's balance index (Kenworthy 1973), Moller–Nielson balance (Nielsen and Friis-Nielsen 1976), crop logging (Abaev 1977), and boundary line

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concept (Walworth, Letzsch, and Sumner 1986). All these diagnosis methods are called univariate methods. They take into consideration only the individual nutrient concentration (Serra et al. 2012). However, the Diagnosis and Recommendation Integrated System (DRIS) and the Compositional Nutrient Diagnosis (CND) are two methods which consider a specific combination of nutrient content allowing the diagnosis of plant nutritional balance (Bhaduri and Pal 2013). Furthermore, the methods that used the nutritional balancing concept are more precise in the detection of nutritional deficiencies or/and excesses compared to the other traditional diagnosis methods (Barłóg, Grzebisz, and Błaszyk 2017; Mourão Filho 2004). In Tunisia, the use of plant analysis as a diagnostic tool is not widespread. In fact, local leaf tissue norms have not been yet developed. For this reason, only a minority of Tunisians farmers use norms that are recommended to them since they have been developed in other countries with varieties and climatic conditions similar to Tunisia. However, Munson, Nelson, and Westerman (1990) indicated that locally or regionally developed norms produce a higher degree of precision in diagnosing deficiencies or imbalances than other regions developed norms. Therefore, this work aimed at studying the relation between yield and foliar nutrient concentration of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), Boron (B) and Zinc (Zn) in mandarins leaves to develop leaf nutrients optimum ranges (NOR) called 'Beaufils ranges' by two different methods "DRIS" and "CND", in order to enhance the diagnosis accuracy of leaf analysis. Moreover, both DRIS and CND methods allowed us to evaluate the efficiency of fertilization practices used by mandarins' growers. Then, the developed norms were compared to the most used norms by growers and agriculture extension services in Tunisia.

Materials and methods

Data

This study was carried out in the period 2011-2014 on commercial orchards selected in several regions of Tunisia that is located between latitude 37°20 '18" N, longitude 09°45′37" E and latitude 30°14 '24" N, longitude 09°37′46" E. In total, 120 observations were conducted in order to ensure that our result represents the current various growing areas and growth conditions. However, the majority of the samples were taken in the northeastern Tunisia in the Cap Bon peninsula, where the principal citrus fruits production centers are implanted due to quite suitable environmental conditions. According to the World Reference Base for Soil Resources (WRB) classification system (Fao 2015), the majority of studied soils have been classified as Luvisols (Alfisols) and Kastanozems (Xerolls). The others belong to calcisols (Calcids), Gypsisols (Gypsids), Leptosols, and Cambisols. Soils are calcareous and fall between the near neutral soils and alkaline soils. Generally, they are sandy soils with low organic matter content and lack nitrogen (N), phosphorus (P), and micronutrients, especially zinc (Zn) and iron (Fe). Each region of Tunisia has a different kind of climate. The north and the coasts have a Mediterranean-type climate, whereas in the interior of the country the climate becomes semi-arid then arid in the southern part.

The data bank to compose the DRIS norms is formed by the total concentration of nine nutrients in the leaves and the yield of mandarins (*Citrus reticulata* Blanco). Mandarin's groves are budded on Sour orange [*Citrusaurantium* (L.)], the main important Tunisian rootstock for citrus. When nutrient levels in leaf tissues are stable, undamaged and exposed leaves from the perimeter of 15 to 20 normal and healthy trees were randomly sampled in each commercial citrus orchard from mid-September through early October. Seven-to-eight -month-old spring flush leaves were taken from the non-fruiting twigs. A sample included a minimum of 50 leaves picked from each side (N-S-E-W) of the trees.

In the lab, leaves were rinsed indistilled water to remove surface contamination, and then oven-dried and grounded. The total N was determined by micro-Kjeldahl method. The leaf samples were prepared for elemental analysis P, potassium (K), calcium (Ca), magnesium (Mg), Fe, copper (Cu), boron (B), and Zn through destruction of organic matter by dry ashing. Then, the concentration of P was determined by reduction with molibdo-vanadate and the concentrations of K, Ca, Mg, Fe, Cu, B, and Zn were determined using atomic absorption spectrometry (model: Thermo Scientific iCE 3500, Thermo Electron Manufacturing Ltd, Cambridge, United Kingdom).

The leaf nutrient concentration must follow a normal distribution (Serra et al. 2012). That is why, the transformation of the data of nutrient concentration from leaf tissue with CND and DRIS methods, by the calculation of row-centered log ratio and the logarithmic transformation, respectively, correct partially the non-normal distribution. In order to ensure normal distribution, outliers are detected in yield and raw compositional nutrients (leaf nutrient concentration) in the database through Mahalanobis distance (Figure 1). Only 60 orchards were retained in the database. The Gaussian distribution was observed using the Shapiro–Wilk test for the CND and DRIS methods.

Partitioning data into high- and low-yielding subpopulations

Based on commercial yield, the orchards were divided into two groups: high-yield orchards rated as reference population and low-yield orchard rated as non-reference population. The yield cutoff for the high-yielding and low-yielding populations was determined following the statistical critical value approach (CVA) of (Cate and Nelson 1971). First, the yield data were arranged in descending order. Starting with the initial yield value, the corrected sum of squares of the two populations that result from moving to each successive yield value were calculated, which is also referred to as R^2. The samples with higher R^2 values than maximum were referred to as the high-yield or reference population (≥95 kg tree^{-1}), whereas the remaining samples were referred as low-yielding population. Sample size selected for the high-yielding population for developing the DRIS norms and CND norms was 33 out of 60 samples.

DRIS norms

For the high-yielding subpopulations, the mean, standard deviation, and variance were calculated for each nutrient concentration as well as for all the dual ratios between nutrient concentrations

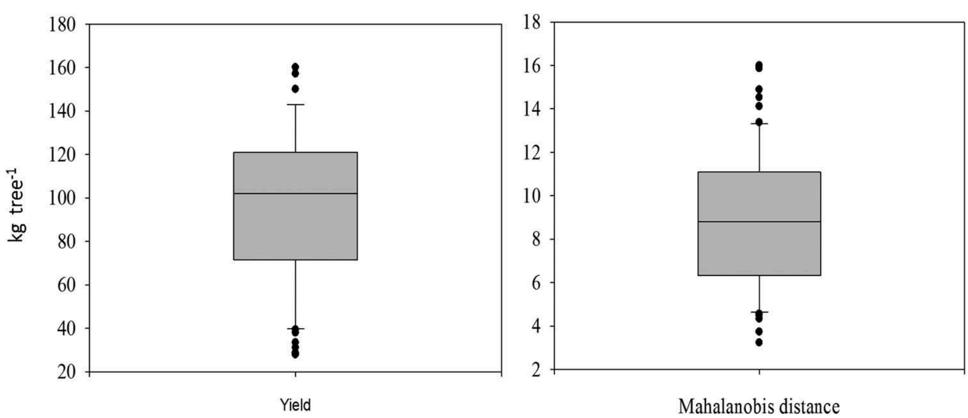

Figure 1. Detection of outliers in yield and raw compositional nutrients (leaf nutrient concentration) in database through Mahalanobis distance.

Table 1. Mean and standard deviation (SD) of DRIS norms.

Ratio	Mean	SD	Ratio	Mean	SD
Ln N/P	3.100	0.231	Ln Mg/Fe	−6.988	0.762
Ln N/K	1.031	0.327	Ln Mg/Cu	−3.345	0.895
Ln N/Ca	−0.684	0.237	Ln Mg/B	−6.264	0.831
Ln N/Mg	2.781	0.581	Ln Mg/Zn	−5.259	0.614
Ln N/Fe	−4.207	0.330	Ln Fe/N	4.207	0.330
Ln N/Cu	−0.564	0.575	Ln Fe/P	7.268	0.217
Ln N/B	−3.482	0.532	Ln Fe/K	5.199	0.263
Ln N/Zn	−2.477	0.674	Ln Fe/Ca	3.483	0.187
Ln P/N	−3.100	0.231	Ln Fe/Mg	6.949	0.582
Ln P/K	−2.069	0.338	Ln Fe/Cu	3.603	0.540
Ln P/Ca	−3.785	0.241	Ln Fe/B	0.685	0.480
Ln P/Mg	−0.319	0.663	Ln Fe/Zn	1.690	0.678
Ln P/Fe	−7.307	0.330	Ln Cu/N	0.564	0.575
Ln P/Cu	−3.664	0.655	Ln Cu/P	3.664	0.655
Ln P/B	−6.583	0.410	Ln Cu/K	1.595	0.623
Ln P/Zn	−5.578	0.781	Ln Cu/Ca	−0.120	0.625
Ln K/N	−1.031	0.327	Ln Cu/Mg	3.345	0.895
Ln K/P	2.069	0.338	Ln Cu/Fe	−3.642	0.663
Ln K/Ca	−1.716	0.365	Ln Cu/B	−2.918	0.756
Ln K/Mg	1.750	0.576	Ln Cu/Zn	−1.913	0.937
Ln K/Fe	−5.238	0.504	Ln B/N	1.244	0.653
Ln K/Cu	−1.595	0.623	Ln B/P	6.583	0.410
Ln K/B	−4.514	0.582	Ln B/K	4.514	0.582
Ln K/Zn	−3.509	0.705	Ln B/Ca	2.798	0.398
Ln Ca/N	0.684	0.237	Ln B/Mg	6.264	0.831
Ln Ca/P	3.785	0.241	Ln B/Fe	−0.724	0.515
Ln Ca/K	1.716	0.365	LnB/Cu	2.918	0.756
Ln Ca/Mg	3.466	0.620	Ln B/Zn	1.005	0.955
Ln Ca/Fe	−3.522	0.294	Ln Zn/N	2.477	0.674
Ln Ca/Cu	0.120	0.625	Ln Zn/P	5.578	0.781
Ln Ca/B	−2.798	0.398	Ln Zn/K	3.509	0.705
Ln Ca/Zn	−1.793	0.711	Ln Zn/Ca	1.793	0.711
Ln Mg/N	−2.781	0.581	Ln Zn/Mg	5.259	0.614
Ln Mg/P	0.319	0.663	Ln Zn/Fe	−1.729	0.779
Ln Mg/K	−1.750	0.576	Ln Zn/Cu	1.913	0.937
Ln Mg/Ca	−3.466	0.620	Ln Zn/B	−1.005	0.955

(Table 1). The method that was tested to compose the DRIS norms was transformation by natural log (NL) of the ratios between the nutrient concentrations (Serra et al. 2012). This method of dual ratio choice was the application of log natural transformation (LN) in the dual ratios and both directly (A/B) and reverse (B/A) dual ratios were selected to compile DRIS norms. According to Jones (1981) DRIS function values of the nutrients from the average values in highly productive population (a/b), were determined, in units of standard deviation (s) using an adjustment factor (c) = 1,

$$f\left(\frac{A}{B}\right) = \left[\left(\frac{A}{B}\right) - \left(\frac{a}{b}\right)\right] \cdot \left(\frac{c}{s}\right)$$

After defining the DRIS functions, a DRIS index for each nutrient is determined, which may have positive or negative values, following the general formula proposed by (Beaufils 1973), being for a nutrient A:

$$DRIS\ indexA = \frac{\sum f\left(\frac{A}{B}\right) - \sum f\left(\frac{B}{A}\right)}{n + m}$$

here n = number of DRIS functions involved in the analysis, in direct form (A/B); m = number of DRIS function included in the analysis, in the inverse form (B/A).

CND norms

For the establishment of CND norms, the proposal of Khiari, Parent, and Tremblay (2001b) was adopted.

$$V_N = \log\left(\frac{N}{G}\right),\ V_P = \log\left(\frac{P}{G}\right), V_K = \log\left(\frac{K}{G}\right), \ldots,\ V_{Rd} = \log\left(\frac{Rd}{G}\right),$$

in which V_N,V_P,V_K,…V_{Rd} = multinutrient variables for the concentration of the nutrients N, P, K,…,Rd (mg kg^{-1}); and G = geometric mean of dry matter constituents. To calculate the geometric mean (G), the following equation was used:

$$G = (NxPxKx\ldots.xR_d)^{\frac{1}{d+1}}$$

in which G = geometric mean of dry matter constituents; N, P, K,…, R_d = concentration of the nutrients in the dry matter (%); R = residue or concentration of the unmeasurable nutrients in the dry matter (%); and d = number of nutrients evaluated.

The means and standard deviations of row-centered log ratios of the nutrients concentration in leaf tissue of the high-yielding subpopulation were denoted as 'V_N + 'V_P + 'V_K + …'V_{Rd} and 'SD_N, 'SD_P, 'SD_K,… 'SD_{Rd}, respectively, were then calculated (Table 2).

The CND indices, denoted as I_N, I_P, I_K,…,I_{Rd}, were calculated from the row-centered log ratios as follows:

$$I_N = \frac{V_N - {'V_N}}{{'SD_N}}, I_P = \frac{V_P - {'V_P}}{{'SD_P}}, I_K = \frac{V_K - {'V_K}}{{'SD_K}}, \ldots\ldots, I_{Rd} = \frac{V_{Rd} - {'V_{Rd}}}{{'SD_{Rd}}}$$

Establishment of NOR

For the interpretation of DRIS and CND index values by the Beaufils ranges, regression equations between foliar nutrient concentrations and indices, by the two methods were traced. The optimal range of nutrients was obtained when the statistical models of the relationship of the nutrient content and the indexes tend to zero ($y = ax + b = 0$). The lower and upper limits of the normal nutrient range for the DRIS and CND were determined analogously to the method used by Beaufils (1973) . According to the standard deviation range (s) of DRIS and CND indices from the reference population, five classes were determined: deficient nutritional status < −4/3 (s); deficiency-prone = −4/3 to −2/3 (s); sufficient = −2/3 to 2/3 (s); tendency to excess = 2/3 to 4/3 (s); excessive levels > 4/3 (s).

Table 2. Mean and standard deviation (SD) of CND-clr norms.

	Mean	SD
VN	2.868	0.162
VP	−0.232	0.233
VK	1.837	0.273
VCa	3.553	0.181
VMg	0.087	0.529
VFe	−2.135	0.328
VCu	−5.778	0.550
VB	−2.860	0.462
VZn	−3.865	0.618
VRd	6.525	0.111

Nutrient application potential response (NAPR)

NAPR was developed to interpret the DRIS index and CND index (Silveira, Nachtigall, and Monteiro 2005). This method compares the index of each nutrient with the value of the mean nutritional balance index to verify if the imbalance attributed to a given nutrient is greater or less than the imbalance attributed to the mean of all nutrients.

This method of interpretation consists on grouping five categories of NAPR (zero"Z", positive or zero "PZ", positive "P", negative or zero "NZ"), reflecting respectively, balanced nutritional status, probable deficiency nutritional status, deficient nutritional status, probable excess nutritional status, and excess nutritional status. In order to define these classes, the rates of each nutrient index (IN) were compared to the nutrient balance index average (NBIa), which is the arithmetic average of the module of all DRIS and CND index (Wadt 1996): (i) "Z" = |IN| < NBIa; (ii) "PZ" with low probability = |IN| > NBIa, with IN < 0; (iii) "P" = |IN| > NBIa, where IN is the lowest index among the other nutrients; (iv) "NZ" with low probability = |IN| > NBIa, where IN >0 and (v) "N" = |IN| > NBIa, where IN is the highest index among the other nutrients. $NBIa = (|IA| + |IB| + |IC| + \ldots + |IN|)/n$

Where IA, IB, IC,., IN = indexes of nutrients A,B,C,..,N; n = the number of evaluated nutrients. A positive response "P" implies that nutrient inputs would increase crop yields, or by improving the quality of fruits and a negative response "N" implies that nutrient inputs would decrease the crop yield and productivity. The balanced status "Z" means that no crop response is expected in relation to the application of the nutrient in the soil.

Statistical analysis

For both methods, a Chi-square test (χ^2) of Pearson was applied at 1% probability, with n-1 degrees of freedom (n = number of analyzed nutrients) in order to test the effectiveness of the nutritional diagnosis. If the observed frequencies for all nutrients are statistically equal, the hypothesis of randomness of the results diagnosed as deficient will be true (Serra et al. 2014, 2012). The Chi-square test (χ^2), expected (*EF*) and observed frequencies (*OF*) were calculated as follows:

$$\mathrm{EF}(\%) = \left[\left(\frac{\text{Total number of plots evaluated}}{\text{Number of nutrients evaluated}}\right)/(\text{Total number of plots evaluated})\right].100$$

$$\mathrm{OF}(\%) = \left(\frac{\text{Total number of plots evaluated as deficient}}{\text{Total number of plots}}\right).100$$

$$\chi^2 = \sum_{i=1}^{k}\left[\frac{(\mathrm{OFi}-\mathrm{EFi})^2}{\mathrm{EFi}}\right]$$

Statistical analysis was performed using the program SPSS for Windows, version 20.0.0. The other DRIS and CND calculations were performed with Excel® (2010) spreadsheets.

Results and discussion

Relationship between leaf nutrient content and DRIS, CND index in high-yielding subpopulation

For both methods, regression equations were fitted to the relationships between nutrient content in leaves and indices. Linear models were adjusted. When comparing the two tested methods, coefficients of determination based on the CND method were a bit higher for Ca, and Fe which may show that there is greater dependency of the DRIS indices on the content of the element itself than on the other nutrients involved in the calculation of DRIS indices as it was mentioned by (Serra et al. 2012) while explaining the reason why the macronutrients had lower coefficients of determination than the micronutrients adopting DRIS method. In spite of slight differences in the coefficients of determination, they all show the same tendency.

These coefficients of determination were between 0.565–0.905, respectively, for the relationship between N, Mg leaf concentrations, and their CND index. However, coefficients of determination using DRIS method were ranged from 0.557 to 0.896, respectively, for the nutrients N and Mg. (Figures 2, 3).

It was noticed that the relationship between the indices for N and the concentration of N using the two methods was the lowest compared to all nutrients. These results are consistent with research

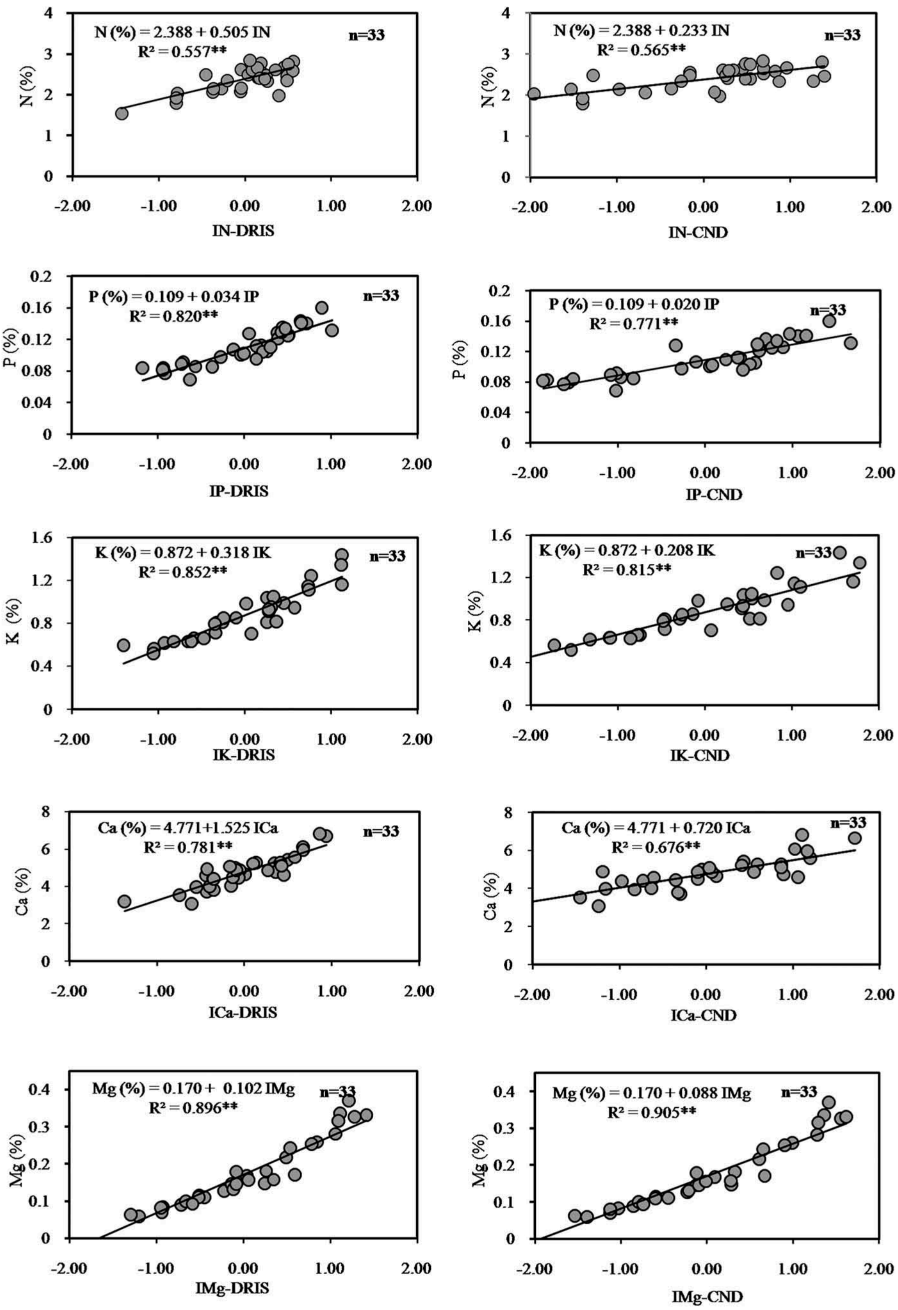

Figure 2. Relationships between the leaf macro nutrient contents in high-yielding subpopulation and DRIS and CND index. **p<0.01

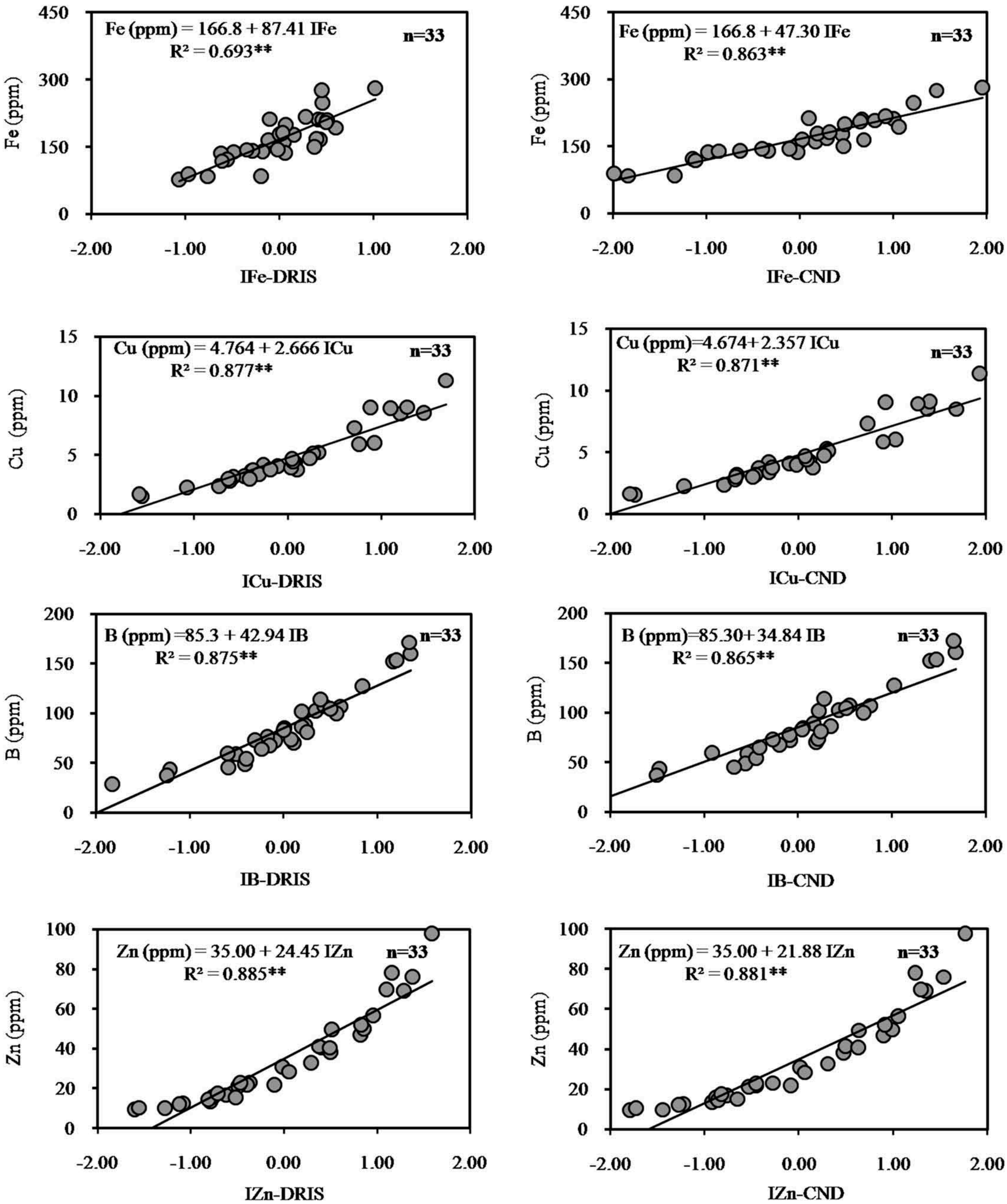

Figure 3. Relationships between the leaf micro nutrient contents in high-yielding subpopulation and DRIS ans CND index. **p<0.01

studies for other crops, such as sugarcane (Guimarães et al. 2015; Junior, Dos Anjos, and Monnerat 2003), cotton (Serra et al. 2012), signal grass (Silveira, Nachtigall, and Monteiro 2005), cherry and hazelnut (Righetti, Alkoshab, and Wilder 1988), apple (Nachtigall and Dechen 2007) and coffee (Silva, Farnezi, and Andrade 2013). It was reported that these results indicate that for nitrogen, indices are strongly dependent on the concentration of the other nutrients in the leaves, while for the

Table 3. Equations relating *Y* as CND index to *X* as DRIS index.

Component	Equation	R^2 value
N	$Y = 2.122X - 0.051$	0.869**
P	$Y = 1.572X - 0.011$	0.983**
K	$Y = 1.479X - 0.003$	0.987**
Ca	$Y = 1.834X - 0.022$	0.931**
Mg	$Y = 1.162X + 0.010$	0.997**
Fe	$Y = 2.044X - 0.012$	0.931**
Cu	$Y = 1.131X + 0.002$	0.997**
B	$Y = 1.210X + 0.002$	0.992**
Zn	$Y = 1.111X + 0.014$	0.996**

**$p < 0.01$.

other nutrients indices are more dependent on their own concentrations (Nachtigall and Dechen 2007; Silveira, Nachtigall, and Monteiro 2005). Despite the lowest coefficient of determination for N, correlations were verified ($p < 0.01$) between the concentrations of nutrients and their respective DRIS and CND indices (Figure 2,3). Similar results were obtained in diagnosing the nutritional status of 'Newhall' navel orange trees (Huang et al. 2012). For N and K nutrients, although the coefficients of determination were the lowest with 0.45 and 0.53, respectively, the optimum models established between all leaf nutrient concentrations and their corresponding DRIS indices were related ($p < 0.01$).

Litter differences between CND and DRIS indexes were found in this study. This fact is in line with findings an annual crops, for example, on tomato (Parent, Karam, and Visser 1993), carrot (Parent et al. 1994) and sweet corn (Khiari, Parent, and Tremblay 2001a), as well as on banana and coffee (Wairegi and van Asten 2011; Wairegi and Van Asten 2012). All regressions relating CND to DRIS were significant ($p \leq 0.01$) (Table 3). The R^2 ranged 0.869–0.997 for relationships between CND and DRIS, for nutrient indices. According to Barłóg (2016), CND indices were also closely related to DRIS indices ($R^2 > 0.93$). Therefore, the two methods seem equally good in diagnosing nutrient imbalances. For example, the order of imbalances in one citrus orchard was Mg (−1.03) < P (−0.27) < B (−0.08) < Fe (−0.02) < Ca (0.12) < Cu (0.12) < Zn (0.30) < K (0.69) < N (0.96) for CND, Mg (−0.93) < P (−0.27) < B (−0.08) < Fe (−0.01) < Ca (0.01) < Cu (0.09) < Zn (0.30) < N (0.44) < K (0.45) for DRIS. Although the order differed between CND and DRIS, the two methods identified Mg as being most deficient, N and K as least deficient. This is suggest that the two approaches did not seem to differ in categorizing of observations as either deficient or excess.

Table 4. Frequency (%) of the N, P, K, Ca, Mg, Fe, Cu, B, and Zn fertilization response potential determined by the DRIS and CND methods in citrus, in the low productivity subpopulation.

Nutrients	Method	*p*	*Pz*	*z*	*nz*	n
N	DRIS	0.00	7.41	88.89	3.70	0.00
	CND	3.70	22.22	55.56	18.52	0.00
P	DRIS	22.22	3.70	55.56	3.70	14.81
	CND	22.22	3.70	51.85	11.11	11.11
K	DRIS	0.00	11.11	44.44	33.33	11.11
	CND	0.00	14.81	48.15	3.70	33.33
Ca	DRIS	14.81	3.70	66.67	11.11	3.70
	CND	14.81	14.81	48.15	7.41	14.81
Mg	DRIS	14.81	3.70	29.63	22.22	29.63
	CND	14.81	0.00	37.04	33.33	14.81
Fe	DRIS	14.81	11.11	70.37	0.00	3.70
	CND	18.52	14.81	51.85	11.11	3.70
Cu	DRIS	3.70	14.81	66.67	11.11	3.70
	CND	3.70	3.70	77.78	14.81	0.00
B	DRIS	7.41	22.22	55.56	7.41	7.41
	CND	3.70	14.81	66.67	11.11	3.70
Zn	DRIS	11.11	3.70	59.26	0.00	25.93
	CND	7.41	7.41	62.96	3.70	18.52

NAPR

According to the NAPR, P and Fe were most frequently diagnosed as deficient with the two methods (Table 4). P and Fe deficiencies were probably due to Tunisian's alkaline and calcareous soils. P availability is limited in calcareous soils. After P fertilizer is applied to these soils, they undergo a series of chemical reactions with Ca which solubility decreases with time (Obreza, Alva, and Calvert 1993). The Fe content in this type of soil could be high, while the availability for the trees is still insufficient. Citrus, deciduous fruit trees, olive, grapevine, and berries were the most important commercial crop affected by Fe deficiency chlorosis (Àlvarez-Fernàndez, Abadía, and Abadía 2006). Iron deficiency chlorosis is a major nutritional problem in sensitive plants growing on calcareous soils. It has been related to the content and reactivity of Fe oxides and the properties of carbonate which buffers pH at alkaline values. The solubility level reaches a minimum in the pH range between 7.4 and 8.5 (Obreza, Alva, and Calvert 1993; Reyes, Del Campillo, and Torrent 2006). A chi-square test (χ2) between expected (EF) and observed frequencies (OF) of plots evaluated as deficient was used in order to test the effectiveness of the nutritional diagnoses performed by the two methods. The chi-square test revealed that the observed frequencies were not found at random (Table 5). The test was effective ($p < 0.01$) to reject the hypothesis that the observed frequencies in all nutrients were statistically equal. That indicates that the methods were sensitive to diagnose the nutritional state of plants. Similar results

Table 5. Chi–square test and values of observed and expected frequencies as deficient nutrients by the DRIS and CND method.

	DRIS			CND		
	OF	EF	$(OF-EF)^2/EF$	OF	EF	$(OF-EF)^2/EF$
N	0.00	11.11	11.11	3.70	11.11	4.94
P	22.22	11.11	11.11	22.22	11.11	11.11
K	0.00	11.11	11.11	0.00	11.11	11.11
Ca	14.81	11.11	1.23	14.81	11.11	1.23
Mg	14.81	11.11	1.23	14.81	11.11	1.23
Cu	3.70	11.11	4.94	3.70	11.11	4.94
Fe	14.81	11.11	1.23	18.52	11.11	4.94
B	7.41	11.11	1.23	3.70	11.11	4.94
Zn	11.11	11.11	0.00	7.41	11.11	1.23
χ2			43.21**			45.68**

[(1)]OF and EF: observed and expected frequencies (%) in low productivity subpopulation, respectively.
**$p < 0.01$

Table 6. Nutrients optimal range determined for the nutritional diagnosis of mandarins based on DRIS norms and CND norms.

Nutrients	Method	Deficient	Tendency To deficiency	Sufficient	Tendency to excess	Excess
N (g/kg)	DRIS	> 19.7	19.7 – 21.8	21.8 – 26.0	26.0 – 28.0	> 28.0
	CND	> 19.7	19.7 – 21.8	21.8 – 26.0	26.0 – 28.0	> 28.0
P (g/kg)	DRIS	> 0.8	0.8 – 0.9	0.9 – 1.2	1.2 – 1.4	> 1.4
	CND	> 0.8	0.8 – 0.9	0.9 – 1.2	1.2 – 1.4	> 1.4
K (g/kg)	DRIS	> 5.6	5.6 – 7.2	7.2 – 10.3	10.3 – 11.8	> 11.8
	CND	> 5.6	5.6 – 7.2	7.2 – 10.3	10.3 – 11.8	> 11.8
Ca (g/kg)	DRIS	> 36.1	36.1 – 41.9	41.9 – 53.6	53.6 – 59.4	> 59.4
	CND	> 36.0	36.0 – 41.9	41.9 – 53.6	53.6 – 59.4	> 59.4
Mg (g/kg)	DRIS	> 0.5	0.5 – 1.1	1.1 – 2.3	2.3 – 2.9	> 2.9
	CND	> 0.5	0.5 – 1.1	1.1 – 2.3	2.3 – 2.9	> 2.9
Fe (mg/kg)	DRIS	> 99	99 – 133	133 – 201	201 – 235	> 235
	CND	> 99	99 – 133	133 – 201	201 – 235	> 235
Cu (mg/kg)	DRIS	> 1.4	1.4 – 3.1	3.1 – 6.4	6.4 – 8.1	> 8.1
	CND	> 1.3	1.3 – 3.0	3.0 – 6.4	6.4 – 8.0	> 8.0
B (mg/kg)	DRIS	> 35	35 – 60	60 – 110	110 – 135	> 135
	CND	> 35	35 – 60	60 – 110	110 – 135	> 135
Zn (mg/kg)	DRIS	> 4	4 – 20.0	20 – 51	51 – 66	> 66
	CND	> 4	4 – 20.0	20 – 51	51 – 66	> 66

were reported in an evaluation of the nutritional status of several species such as Eucalyptus, soybean, cotton crops, and orange trees (Hernandes et al. 2014; Serra et al. 2014, 2012).

Comparison between NOR and other nutrient standards

For citrus, Beverly (1987) was the first researcher who developed preliminary DRIS norms to diagnose nutritional status of N, P, K, Ca, and Mg for "Valencia" sweet orange in California, USA. Subsequently, there was a lot of research about the application of DRIS method in citrus cultivars. Different NOR were carried out in different regions such as China, India, and Brazil (Huang et al. 2012; Mourão Filho and Azevedo 2003; Srivastava, Singh, and Tiwari 2007). These standards are different from the norms, which were developed in this study (Table 6). DRIS norms differ widely as per the diversity in citrus-growing regions. Srivastava and Alila (2006) suggested leaf nutrient norms for mature "Khasi" mandarin with yield 32–56 kg tree^{-1} in northeast India. Leaves are 6- to 7-month old taken at second, third, or fourth leaf positions from non-fruiting terminals. They recommended optimum ranges for different, while (Srivastava and Singh 2008) developed a leaf nutrient standards for "Nagpur" mandarin in Central India derived from spring-cycle leaves (6–8 months old) from non-fruiting terminals in relation to fruit yield of 47.7–117.2 kg tree^{-1}. These limits turn out to be widely different in China using optimum values obtained with critical nutrient concept, for Satsuma mandarin using third leaf from vegetative terminals (Wang 1985).

However, NOR involving N, K and Ca established in this research (Table 6) were quite comparable to the previously determined in the literature by Quiñones et al. (2012) applying Kenworthy's balance index procedure for mandarin orchards in east of Spain (Comunidad Valenciana) which 7 to 9-month-old leaves were collected from new spring flushes without terminal fruit. These authors recommended an optimum range of 24.1–27.0 g kg^{-}1 N, 7.1–10.0 g kg^{-1} K and 30–50 g kg^{-1} Ca. Regarding sufficiency ranges in the case of N, the determined ranges by the two methods (21.8–26.0 g kg^{-1} N) was slightly wider than the sufficiency range in Spain and most of the latter is included inside the range. Otherwise, the new recommended range (41.9–53.6.2 g kg^{-1}) for Ca was narrower than the corresponding recommended range in Spain and the latter covers entirely the new recommended sufficiency range. Furthermore, our determined sufficiency range was nearly identical to those founded by Raveh (2013) with another interpretation tool "boundary line approach "in mature mandarin orchards which sufficiency range of 20–24 g kg^{-1} N, 0.9–1.2 g kg^{-1} P, 5.5–6.9 g kg^{-1} K,1.9–2.6 g kg^{-1} Mg. Conversely to our research, these mineral values in those guidelines are related to spring-flush leaves that are 4 to 6 months old, sampled from fruiting twigs. There are differences between leaf nutrient concentration from fruiting and nonfruiting branches, reflecting the sink strength of the fruit. Khan, Srikandakumar, and Embleton (2001) were reported that N, P, K, Fe, Cu, Mn and B were significantly higher in nonfruiting leaves and Ca, Mg and Cl were significantly higher in fruiting leaves. This agrees with our observation. N and K optimal range recommended by Raveh (2013) were small lower than those found in this case, apart from P where optimal nutrient ranges were in good agreement. While, Mg optimal range was higher than the optimal nutrient range founded. These similarities were probably due to similar Mediterranean climate conditions, soil fertility, and varieties. The main characteristic of the Mediterranean climate is that it has two well-defined seasons in the year, with the rain period coinciding with low temperatures (winter) while summers are hot and almost completely dry. The soils developed over carbonaceous rocks which are the most important parent material in Mediterranean areas and thus the soil is calcareous (pH > 7) due to the presence of excess calcium carbonate ($CaCO_3$) or magnesium carbonate ($MgCO_3$) (Rashid and Ryan 2004).

Simulation of nutrients optimal range involving populations grouped by several management methods applied cannot be universally applied (Hernandes et al. 2014). For sugarcane growing in different climatic and soil conditions in South Africa, United State, and Brazil, it was shown that it is necessary to develop specific norm for each region (Junior, Dos Anjos, and Monnerat 2003).

DRIS norms were developed for 'Valencia' sweet orange for a plant population with different plant ages, on various rootstocks, at several regions for the four most important citrus-producing states of Venezuela. It was found that there is good agreement between all sets of "Volckameriana lemon" and "Cleopatra tangerine" rootstock norms indicating that type of rootstock does not have influence on the norms derived (Rodríguez, Rojas, and Sumner 1997). NOR should be developed in specific conditions in which many factors correlating with yield or quality are known: cultivar, rootstock, climate, soil, and crop management productivity. Irrespective of some physiographical divergence, norms developed in one specific region, if applied to another region, the elemental composition of high yielding orchards needs to be nearly identical (Srivastava and Singh 2008).

Conclusions

The CND and DRIS methods were effective to diagnose the plant nutritional status. The diagnoses were not random based on the effectiveness of the chi-square-tested method. The use of the DRIS and CND methods generates equal estimates for all nutrients. Thus, both methods can be used to develop nutrients optimal ranges and diagnose the nutritional mandarin states.

NOR for mandarins (*Citrus reticulata* Blanco) in Tunisia have been derived for the first time using nonfruiting shoot leaves, from the spring flushes (7- to 8-month old).DRIS and CND indexes developed for mandarins cultivars in this study predicted optimum value of different nutrients as: 21.8–26.0 g kg^{-1} N, 0.9–1,2 g kg^{-1}P, 7.2–10.3 g kg^{-1} K, 41.9–53.6 g kg^{-1} Ca, 1.1–2.3 g kg^{-1} Mg, 133–2.1 ppm Fe, 3.1–6.4 ppm Cu, 60–110 ppm B and 20–51 ppm Zn, in relation to fruit yield of 95–160 kg tree $^{-1}$.

They are likely to provide the desired guidance to add sustainability in production much better than before because the new found norms were based on the local varieties that were growing under the local climate and soil conditions. The study also concludes that both CND and DRIS can be used to determine nutrient imbalances. The P and Fe showed the higher frequency as the most required nutrients by plant due to the alkaline/calcareous Tunisian soil. To be effective on this type of soil, P fertilizer should be applied in water-soluble form during the vegetative stage where the plant needs it. whereas, Fe chlorosis problem can be resolved by applying Fe chelates in EDDHA form which has been shown to be effective for Tunisian type of soil (Obreza, Alva, and Calvert 1993). Moreover, foliar applications are helpful in correcting the deficiency of Fe on fruit trees. To conclude, the present study has allowed local growers adopting new norms to improve their fertilization practices and consequently their mandarins' productivity because the foliar analysis would be more precise and represent better the mineral status of the tree.

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References

Abaev, M. 1977. Some results of studies on foliar diagnosis of lemon trees. *Subtropicheskie Kultury* 5 (6):150–53.

Àlvarez-Fernàndez, A., J. Abadía, and A. Abadía. 2006. Iron deficiency, fruit yield and fruit quality. In *Iron nutrition in plants and rhizospheric microorganisms*, 1st ed. 85–101. Netherlands: Springer.

Barłóg, P. 2016. Diagnosis of sugar beet (beta vulgaris l.) nutrient imbalance by dris and cnd-clr methods at two stages during early growth. *Journal of Plant Nutrition* 39 (1):1–16. doi:10.1080/01904167.2014.964366.

Barłóg, P., W. Grzebisz, and R. Błaszyk. 2017. Compositional nutrient diagnosis in sugar beet with sodium supplementation. *Archives of Agronomy and Soil Science* 63 (6):856–69. doi:10.1080/03650340.2016.1240364.

Beaufils, E. 1973. Diagnosis and recommendation integrated system (dris). A general scheme of experimentation and calibration based on principles developed from research in plant nutrition. 132 p. *University of Natal, Pietermaritzburg, South Africa.* .

Beverly, R. 1987. Fertilizer use efficiency: Modified dris method for simplified nutrient diagnosis of 'valencia'oranges. *Journal of Plant Nutrition* 10 (9–16):1401–08. doi:10.1080/01904168709363672.

Bhaduri, D., and S. Pal. 2013. Diagnosis and recommendation integrated system (dris): Concepts and applications on nutritional diagnosis of plants–A review. *Journal of Soil and Water Conservation* 12 (1):70–79.

Cantarella, H., J. Quaggio, O. Bataglia, and B. Van Raij. 1992. Response of citrus to npk fertilization in a network of field trials in são paulo state, brazil. *Proceedings of International Society of Citriculture* (2): 607–612

Cate, R. B., and L. A. Nelson. 1971. A simple statistical procedure for partitioning soil test correlation data into two classes 1. *Soil Science Society of America Journal* 35 (4):658–60. doi:10.2136/sssaj1971.03615995003500040048x.

Fao, I. 2015. Status of the world's soil resources (swsr)–Main report. *Food and Agriculture Organization of the United Nations and Intergovernmental Technical Panel on Soils, Rome, Italy*

Guimarães, F. C. N., A. P. Serra, M. E. Marchetti, S. C. Ensinas, P. H. Altomar, V. Do Amaral Conrad, D. C. Potrich, C. B. C. J. Rosa, M. A. Martinez, and F. A. Matos. 2015. Nutrients optimum range (nor) based on dris method to assess the nutritional status of the first ratoon sugarcane. *Australian Journal of Crop Science* 9 (7):638.

Hernandes, A., H. A. de Souza, D. A. de Amorim, W. Natale, J. Lavres Jr, A. E. Boaretto, and M. A. Camacho. 2014. Dris norms for pêra orange. *Communications in Soil Science and Plant Analysis* 45 (22):2853–67. doi:10.1080/00103624.2014.956933.

Huang, H., C. Hu, Q. Tan, X. Zhao, X. Sun, X. Hu, L. Peng, and C. Chun. 2012. Diagnosing of the nutritional status of 'newhall'navel orange trees with the method of modified diagnosis and recommendation integrated system (m-dris). *Journal of Food, Agriculture & Environment* 10 (3):379–83.

Jones, C. 1981. Proposed modifications of the diagnosis and recommendation integrated system (dris) for interpreting plant analyses. *Communications in Soil Science & Plant Analysis* 12 (8):785–94. doi:10.1080/00103628109367194.

Junior, R., R. Dos Anjos, and P. H. Monnerat. 2003. Norms establishment of the diagnosis and recommendation integrated system (dris) for nutritional diagnosis of sugarcane. *Pesquisa Agropecuária Brasileira* 38 (2):277–82. doi:10.1590/S0100-204X2003000200015.

Kalai, L., M. Mnari-Hattab, N. Sadfi, and R. Mohamed Rabeh Hajlaoui. 2010. Caractérisation de l'agent causal du mal secco de l'oranger et évaluation de l'antagonisme bactérien vis-à-vis du phoma tracheiphila. *Biologia Tunisie* 2 (7):30–36.

Kenworthy, A. 1973. Leaf analysis as an aid in fertilizing orchards. *Soil Testing and Plant Analysis Soil Sciences Social Amer Madison* WI:381–92.

Khan, I., A. Srikandakumar, and T. Embleton. 2001. Modelling conversion factors for leaf analyses from fruiting and nonfruiting terminals of citrus to asses the nutrient status of trees. In : Horst W.J. et al. (eds) *Plant nutrition*, Developments in plant and soil sciences, vol 92 724–25. Dordrecht: Springer

Khiari, L., L.-E. Parent, and N. Tremblay. 2001a. Critical compositional nutrient indexes for sweet corn at early growth stage. *Agronomy Journal* 93 (4):809–14. doi:10.2134/agronj2001.934809x.

Khiari, L., L.-E. Parent, and N. Tremblay. 2001b. Selecting the high-yield subpopulation for diagnosing nutrient imbalance in crops. *Agronomy Journal* 93 (4):802–08. doi:10.2134/agronj2001.934802x.

Menino, R. 2012. Leaf analysis in citrus: Interpretation tools. In: Strivastava A. (eds) *Advances in citrus nutrition*, 1st ed. 59–79. Dordrecht: Springer.

Mourão Filho, F. D. A. A. 2004. Dris: Concepts and applications on nutritional diagnosis in fruit crops. *Scientia Agricola* 61 (5):550–60. doi:10.1590/S0103-90162004000500015.

Mourão Filho, F. D. A. A., and J. C. Azevedo. 2003. Dris norms for'valencia'sweet orange on three rootstocks. *Pesquisa Agropecuária Brasileira* 38 (1):85–93. doi:10.1590/S0100-204X2003000100012.

Munson, R. D., W. L. Nelson, and R. Westerman. 1990. Principles and practices in plant analysis. *Soil Testing and Plant Analysis* Ed. 3:359–88.

Nachtigall, G. R., and A. R. Dechen. 2007. Dris norms for evaluating the nutritional state of apple tree. *Scientia Agricola* 64 (3):282–87. doi:10.1590/S0103-90162007000300011.

Nielsen, J. M., and B. Friis-Nielsen. 1976. Evaluation and control of the nutritional status of cereals. *Plant and Soil* 45 (2):317–38. doi:10.1007/BF02372523.

Obreza, T., and K. Morgan. 2008. *Nutrition of florida citrus trees*. second ed. UF/IFAS SL: Florida.

Obreza, T. A., A. K. Alva, and D. V. Calvert. 1993. *Citrus fertilizer management on calcareous soilsed*. Cooperative Extension Service, University of Florida, Institute of Food and Agricultural Sciences: Florida.

Parent, L., A. Karam, and S. Visser. 1993. Compositional nutrient diagnosis of the greenhouse tomato. *HortScience* 28 (10):1041–42.

Parent, L., D. Isfan, N. Tremblay, and A. Karam. 1994. Multivariate nutrient diagnosis of the carrot crop. *Journal of the American Society for Horticultural Science* 119 (3):420–26.

Parent, L., and M. Dafir. 1992. A theoretical concept of compositional nutrient diagnosis. *Journal of the American Society for Horticultural Science* 117 (2):239–242.

Quiñones, A., B. Martínez-Alcántara, E. Primo-Millo, and F. Legaz. 2012. Fertigation: Concept and application in citrus. In : Strivastava A. (eds) *Advances in citrus nutrition*, 281–301. Dordrecht: Springer Netherlands.

Rashid, A., and J. Ryan. 2004. Micronutrient constraints to crop production in soils with mediterranean-type characteristics: A review. *Journal of Plant Nutrition* 27 (6):959–75. doi:10.1081/PLN-120037530.

Raveh, E. 2013. Citrus leaf nutrient status: A critical evaluation of guidelines for optimal yield in israel. *Journal of Plant Nutrition and Soil Science* 176 (3):420–28. doi:10.1002/jpln.v176.3.

Reyes, J., M. Del Campillo, and J. Torrent. 2006. Soil properties influencing iron chlorosis in grapevines grown in the montilla-moriles area, southern spain. *Communications in Soil Science and Plant Analysis* 37 (11–12):1723–29. doi:10.1080/00103620600710470.

Righetti, T. L., O. Alkoshab, and K. Wilder. 1988. Verifying critical values from dris norms in sweet cherry and hazelnut. *Communications in Soil Science & Plant Analysis* 19 (13):1449–66. doi:10.1080/00103628809368026.

Rodríguez, O., E. Rojas, and M. Sumner. 1997. Valencia orange dris norms for venezuela. *Communications in Soil Science & Plant Analysis* 28 (15–16):1461–68. doi:10.1080/00103629709369887.

Serra, A. P., M. E. Marchetti, E. P. Rojas, and A. C. T. Vitorino. 2012. Beaufils ranges to assess the cotton nutrient status in the southern region of mato grosso. *Revista Brasileira de Ciência do Solo* 36 (1):171–82. doi:10.1590/S0100-06832012000100018.

Serra, A. P., M. E. Marchetti, S. C. Ensinas, H. S. de Morais, V. Do Amaral Conrad, F. C. N. Guimarães, and G. P. de Oliveira Barbosa. 2014. Diagnosis and recommendation integrated system (dris) to assess the nutritional state of cotton crop in brazil. *American Journal of Plant Sciences* 5 (04):508. doi:10.4236/ajps.2014.54065.

Silva, E., M. Farnezi, and N. Andrade. 2013. Dris norms and critical nutrients: Ranges for coffee beverage quality in high jequitinhonha valley, brazil. *EJBS, Peshawar* 6:39–44.

Silveira, C. P., G. R. Nachtigall, and F. A. Monteiro. 2005. Testing and validation of methods for the diagnosis and recomendation integrated system for signal grass. *Scientia Agricola* 62 (6):520–27. doi:10.1590/S0103-90162005000600002.

Srivastava, A., and P. Alila. 2006. Leaf and analysis interpretation in relation to optimum yield of khasi mandarin (citrus reticulata blanco). Tropical Agricultural Research and Extension 9:8–16.

Srivastava, A., R. Kohli, H. Dass, A. Huchche, L. Ram, and S. Singh. 1999. Evaluation of the nutritional status of nagpur mandarin (citrus reticulata blanco) by foliar sampling. *Tropical Agriculture-London Then Trinidad-* 76 (2):93–98.

Srivastava, A., and S. Singh. 2008. Dris norms and their field validation in nagpur mandarin. *Journal of Plant Nutrition* 31 (6):1091–107. doi:10.1080/01904160802115359.

Srivastava, A., S. Singh, and K. Tiwari. 2007. Diagnostic tools for citrus: Their use and implications in india. *Better Crops–India* 1 (1):26–29.

Terblanche, J., and S. Du Plessis. 1992. Summary of workshop on leaf and soil analysis as a tool for determining fertilizer requirements of citrus. In*: E.T. Ribulato, A. Gentile and G. Refergiato (eds) Proceedings of the International Society of Citriculture : Acireal, Italy.*.

Wadt, P. G. S. 1996. *Os métodos da chance matemática e do sistema integrado de diagnose erecomendação (dris) na avaliação nutricional de plantios de eucalipto.* Viçosa: Universidade Federal de Viçosa.

Wairegi, L., and P. van Asten. 2011. Norms for multivariate diagnosis of nutrient imbalance in the east african highland bananas (musa spp. Aaa). *Journal of Plant Nutrition* 34 (10):1453–72. doi:10.1080/01904167.2011.585203.

Wairegi, L., and P. Van Asten. 2012. Norms for multivariate diagnosis of nutrient imbalance in arabica and robusta coffee in the east african highlands. *Experimental Agriculture* 48 (3):448–60. doi:10.1017/S0014479712000142.

Walworth, J., W. Letzsch, and M. Sumner. 1986. Use of boundary lines in establishing diagnostic norms1. *Soil Science Society of America Journal* 50 (1):123–28. doi:10.2136/sssaj1986.03615995005000010024x.

Wang, T. 1985. Application of fertilizers to satsuma based on leaf analysis. *Journal Soil Sciences (Turang Tongbo)* 16:275–77.