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Proposition of critical levels and nutrient sufficiency ranges in leaves of 'White Moscato' (*Vitis vinifera* 'Muscat') and 'Bordeaux' (*Vitis labrusca* 'Ives')

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Summary

The Compositional Nutrients Diagnosis (CND) can establish indexes that establish deficiency, normality or excess, and even critical levels (CL) or sufficiency ranges (SR) in grapevine leaves. However, this information is scarce in 'White Moscato' and 'Bordeaux' cultivars cultivated in subtropical regions of the world. The study aimed to propose the CND, CL and SR indexes of nutrients in leaves of 'Bordeaux' and 'White Moscato' grapevine cultivars, cultivated in subtropical conditions. Leaves were collected from 105 'White Moscato' and 'Bordeaux' vineyards. Leaves were prepared, dried, ground and subjected to chemical nutrient analysis. Productivity was evaluated. The nutritional status of the grapevine was calculated using the CND method. The CND-r^2 indexes were effective in establishing the nutritional status of 'White Moscato' and 'Bordeaux' grapevines, in relation to the concentration of nutrients in leaves of N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn in deficient, adequate and excessive concentrations. The application of the CND method in the grapevine database showed lower SRs for macronutrients N, K, Ca, Mg and S, and the breadth of the nutritional range for nutrients N, K, Mg, and Fe was smaller than reported in literature. The CND methodology established the critical level and nutrient sufficiency ranges suitable under current grapevine production conditions. Multi-nutrient combinations were more effective than the analysis of a single nutrient in expressing that the limitation of a certain element can reduce the productivity of the grapevines.

Keywords

Leaf analysis, Compositional Nutrients Diagnosis (CND), nutritional balance, fertilization, vineyards.

Introduction

When soils do not have the sufficient amount of nutrients to meet the demand of grapevine cultivars, it is necessary to carry out nutrient applications. However, for the decision-making over the application to be more accurate, it is necessary to define the critical levels (CL) of nutrients in the soil (Brunetto *et al.* 2020, Stefanello *et al.* 2020). But, in grapevines, soil nutrient contents are not always related to the nutrient contents in leaves and neither with the productivity and wine composition variables of the must (Khalil *et al.* 2018, Quiroga *et al.* 2017, Brunetto *et al.* 2020, Stefanello *et al.* 2020). This is because plants such as grapevines may have roots that absorb nutrients in deeper soil layers than those considered diagnostic (e.g., 0-20 cm) (Carranca *et al.* 2018, Mimmo *et al.* 2018); they may have mechanisms that enable the solubilization/availability of forms of nutrients that were initially considered unavailable (non-exchangeable), as in the case of K (Ciotta *et al.* 2021); they tend to absorb nutrients throughout the year, especially in the edaphoclimatic conditions of countries with tropical and subtropical climate and have internal reserves of nutrients, especially in perennial organs, such as roots, branches older than one year and stem (Brunetto *et al.* 2014).

Thus, the analysis of nutrients in grapevine organs, such as in leaves, becomes a technical parameter to assist in deciding the need for nutrient application, because it is the most photosyn-

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thetically activated organ in the plant and the concentration of nutrients in leaves tends to present a relationship with productivity parameters and variables of the oenological composition of the must (Prado and Rozane 2020, Stefanello *et al.* 2021). But univariate methods, such as the CL of a single nutrient in leaves, from which the probability of a plant response is null, do not always relate/correlate with the production or variables of the oenological composition of the must because it does not consider the interactions that occur between other nutrients (Labaied *et al.* 2020, Prado and Rozane 2020). Therefore, it is more appropriate to obtain CL or sufficiency ranges (SR), by considering the relationship between nutrients, two by two (bivariate approach), as done in the DRIS (Diagnosis and Recommendation Integrated System) (Beaufils 1973). But this can also be achieved in a more robust and reliable way, through multivariate methods, such as CND (Compositional Nutrients Diagnosis) (Parent and Dafir 1992, Parent 2011). The CND establishes relationships between the concentration of an element and the geometric mean of the other dry matter components (multivariable relationships), indicating in a more complete way the nutritional balance of the plant (Parent and Dafir, 1992). Through the CND also it is possible to define the CL or SR for adequate grape yields (Rozane *et al.* 2020) or even to establish the values of deficiency, normality or excess (Brunetto *et al.* 2020).

The CND methodology has already been proposed for some wine cultivars in the world (Kumar *et al.*, 2003, Rozane *et al.* 2020). However, studies are still scarce for a larger number of wine cultivars, such as White Moscato (*Vitis vinifera* 'Muscat'), but also for American cultivars such as 'Bordeaux' (*Vitis labrusca* 'Ives'), especially those cultivated under subtropical conditions of the world, where their grapes can be used to make wines, juices, sparkling wines or even other by-products (Ali *et al.* 2010, Kambiranda *et al.* 2020). With the information obtained from the CND, it might be possible to define with greater precision the real need for nutrient application in grapevines, such as in 'Bordeaux' and 'White Moscato' cultivars, which avoid the excess of nutrients in vineyard soils, which can enhance the transference and contamination of surface water adjacent to vineyards (Brunetto *et al.* 2013, Serio *et al.* 2018, Brunetto *et al.* 2020). But it also reduces the cost of acquiring fertilizers and contributes to maintaining and increasing the productivity of grapevines.

In this context, the multinutrient combination (CND method) might be more effective than the analysis of a single nutrient to perform the nutritional diagnosis of vines, and the reference values are more accurate when obtained from regional databases. Thus, the study aimed to propose and compare the CND, CL and SR indexes of nutrients in leaves of 'Bordeaux' and 'White Moscato' grapevine cultivars, cultivated in subtropical conditions.

Material and Methods

Study place

For the study, 105 vineyards were selected in the municipality of Farroupilha, located in the Mesoregion of Rio Grande do Sul Northeast and in the Microregion of Caxias do Sul (latitude 29º13'25" S and longitude 51º20'54" W and altitude 768 m), state of Rio Grande do Sul, southern Brazil. According to Köppen's classification, the climate is Cfb, temperate, with mild summer, well-distributed rainfall and no dry season (Alvares *et al.* 2013). The lowest temperatures occur in the months of June and July, with an average minimum temperature of 8.8°C. In January, the highest temperatures are recorded, with an average maximum temperature of 27.5°C. The average annual precipitation is 1,767.5 mm (IRGA, 2021).

Fifty-one vineyards were from cultivar 'Bordeaux' (*Vitis labrusca* L.) and 54 vineyards were from cultivar 'White Moscato' (*Vitis vinifera* L.). Seventy-two vineyards had grafted grapevines predominantly on the Paulsen 1103 cultivar (*Vitis berlandieri* × *Vitis rupestris*). The rest of the vineyards had grapevines grafted onto cultivars traditionally used in the region, called "white or red horse", and few areas with free standing plants (seedlings produced from cuttings of the crown cultivar) of the 'Bordeaux' cultivar. The age of the vineyards ranged from 2 to 52 years, with 92 vineyards being managed in the trellis system; 9 vineyards in the lyre or manger system and 4 vineyards in the espalier system. The spacing between rows in the vineyards ranged from 1.75 m to 3.8 m and the spacing between plants ranged from 0.93 m to 2.83 m. The soils were either Entisol or Inceptisol classes (Soil Survey Staff 2014).

Leaf sampling and grape production

From each vineyard, 25 leaves were collected in five randomly chosen grapevines (five leaves per grapevine). The leaves were joined to form a sample per vineyard. The complete leaves (limb + petiole) were collected in the middle third of the branch of the year (Brunetto *et al.* 2016). Leaves were collected at full bloom. The leaves did not have mechanical damage or attack from pests and diseases. Immediately after collection, the leaves were dried in a forced air circulation oven at 60°C ± 5°C until constant mass, ground in a Willey mill and passed through a sieve with a mesh of 0.841 mm.

Five grapevines were chosen at random from each vineyard, as was indicated for fruit trees (Oliveira *et al.* 2020) and grapevines (Stefanello *et al.* 2020). In these grapevines, all the bunches of grapes were collected and weighed on a digital scale, for productivity assessment.

Nutrient analysis in leaves

A part of the reserved tissue was digested in sulfuric digestion (Tedesco *et al.* 1995), using a digester block (Tecnal, Micro 42, Piracicaba, Brazil). Nitrogen was determined in a Kjeldahl drag steam still (TE-0364, Tecnal, Piracicaba, Brazil). The other part of the tissue was subjected to nitroperchloric digestion (Tedesco *et al.* 1995). In the extract, the concentrations of Ca, Mg, Cu, Zn, Fe and Mn were determined by an atomic absorption spectrophotometer (AAS; Perkin Elmer, Waltham, MA, USA, AAnalyst 200) (Tedesco *et al.*, 1995). The P concentration was determined by colorimetry, based on the methodology described by Murphy and Riley (1962), in a spectrophotometer (SF325NM, Bel Engineering, Monza, Italy). The concentration of K in the extract was determined in a flame photometer (B262 Micronal, São Paulo, Brazil). The concentration of B was determined by burning 0.5 g of plant tissue sample in the muffle (600°C for 1 h). After cooling, 10 ml of H_2SO_4 0.18 mol l-1 was added, stirred intermittently for 1 hour, staying still to decant for 3 hours. Then, 4 ml of the supernatant was removed and 4 ml of azomethine-H buffer was added, and the determination was carried out at 435 nm in a spectrophotometer (SF325NM, Bel Engineering, Monza, Italy) (Krug *et al.* 1981).

Calculations and statistical analysis

When the data from 105 commercial vineyards of cultivars 'White Moscato' (*Vitis vinifera*) and 'Bordeaux' (*Vitis labrusca*) was analyzed, it was observed that the yield ranged from 60 t ha^{-1} to 3 t ha^{-1}, average yield of 22.3 t ha^{-1} and standard deviation of 11.5 t ha^{-1}. Thus, after excluding four discrepant pieces of data, or outliers (a point that is very far from the other observations in a data distribution) by the Mahalanobis distance (Parent *et al.*, 2009), we performed the evaluation of the normality of the productivity variable using the Kolmogrov-Smirnov test (KS = 0.1045; p>0.20) as indicated by Hair *et al.* (2005). Therefore, H0, that is, the data have a normal distribution.

Raw experimental data are collected in the real space between (-) infinity and (+) infinity. On the other hand, compositional data are strictly positive and provide relative information (Aitchison, 1982). In compositional analysis, leaf tissue is considered to be a 100% closed system, formed by known components (N, P, K, ...) and by unknown components (other undetermined elements, carbohydrates, etc.), brought together in a term called R. That composes a d -dimensional nutrient arrangement, that is, a simplex (S_d) (Equation 1) from the proportions of d + 1 nutrients that include the elements d and a residual value (R_d) (Equation 2):

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

Where: N, P, K.. . are the proportions of nutrients determined in dry matter and R_d is calculated by difference, as follows:

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

Nutrient proportions change to an invariant scale after they are divided by the geometric mean (G) (Equation 3) of *d* +1 components, including R_d (Aitchison 1982):

$$G = [N * P * K * \dots * R_d] \quad (3)$$

To express each component of the *simplex*, in relation to all the others (interaction study), it is sufficient to define new variables (V) (Equation 4), which undergo centered logarithmic transformation (natural or neperian logarithm), that is, in relation to geometric mean of the observed values, being expressed:

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

and, by definition

$$V_N + V_P + V_K + \dots V_{R_d} = 0$$

Thus, considering a single standard deviation, each nutrient has a single relative position in relation to all the others. The advantage of V_x variables is the access to recent multivariate analysis tools, such as principal component analysis and compositional analysis.

The next step is to divide the database into two subpopulations (high and low productivity), using the Cate-Nelson procedure, since the observations were classified in decreasing order of yield, as indicated by Khiari *et al.* (2001a).

In the CND norms, the means and the standard deviation that correspond to the centered logarithmic transformation ratios of V_x of *d* nutrients for specimens with high yields are used, that is $V_N^*, V_P^*, V_K^*, V_R^*$, e $SD_N^*, SD_P^*, SD_K^*, \dots SD_R^*$, respectively.

Once the CND standards have been developed, the independent database can be validated, as reported by Parent and Dafir (1992) serving diagnostic purposes. The CND indexes for *d* nutritive elements are calculated (Equation 5):

$$I_N = \frac{(V_N - V_N^*)}{SD_N^*},\ I_N = \frac{(V_P - V_P^*)}{SD_P^*},\ I_N = \frac{(V_K - V_K^*)}{SD_K^*},\ \dots,\ I_N = \frac{(V_R - V_R^*)}{SD_R^*} \quad (5)$$

Where: V_X^* and S_X^* are the mean and standard deviation of element *X* in the high-yield subpopulation and I_X is the CND index of element *X*.

The independence between the data is guaranteed by the centered logarithmic transformation (Aitchinson 1986). CND indexes are normalized and variables made linear as dimensions of a circle (*d* + 1 = 2), a sphere (*d* + 1 = 3), or a large sphere (*d* + 1 > 3) in a dimensional space of *d* +1. The nutritional imbalance index r^2 is distributed as a variable x_d^2 if the CND indexes are reduced independent variables (Ross 1987), calculated by the equation (Equation 6):

$$r^2 = I_N^2 + I_P^2 + I_K^2 + \cdots + I_R^2 \quad (6)$$

Its radius *r*, computed in the CND nutrient index, thus characterizes each specimen.

To find the FS of each nutrient, after the nutrient index (In) = 0 (balance point = BP) was equated; (BPIn0) was added with (BPIn0) 2/3 of the standard deviation of the nutrient content in the reference population (Kurihara *et al.* 2013, De Souza *et al.* 2015). The NC corresponds to the BP of the FS.

The intensity of the correlation of the coefficients was established considering the classification ranges proposed by Dancey and Reidy (2006). According to classification values from 0.10 – 0.30 are considered to have a weak degree of correlation; 0.40 – 0.60 is moderate and 0.70 – 1.0 is strong.

Results

The database was constituted with information on the concentrations of nutrients in leaves and grape productivity obtained in 101 vineyards, where the grapes are destined for the elaboration of wines, juices, sparkling wines and derivatives. The grape yield variation in the vineyards ranged from 60,331 kg ha^{-1} to 2,994 kg ha^{-1}, the mean was 22,321 kg ha^{-1} and the standard deviation was 11,538 kg ha^{-1}.

Pearson's linear correlation was used to explore the results between concentrations of different nutrient in leaves and also grape yield (Table 1). Among the 66 correlations, 12 were significant. The strong correlation was only observed between Mn-S. Correlations between Ca-Mg, Mg-S, S-Zn, Mn-Zn, Mg-Cu, Mg-Mn and Zn-productivity were classified as moderate (Table 1).

The inflection point of the cumulative productivity function was 21,353 kg ha^{-1}. Vineyards with productivity above this value were classified as the high-yielding subpopulation (reference population). Considering a total of 101 vineyards, 53 of them (52.5%) presented productivity above 21,353 kg ha^{-1} and formed the high productivity subpopulation. The remaining vineyards, 48 (47.5%) formed the low productivity subpopulation (Fig. 1).

After separating the vineyards between high and low productivity, new correlation matrices were created with data on nutrient concentrations in leaves and productivity of each population (Table 2). In the high productivity population, 18 significant correlations were obtained (Table 1), one being strong, eight moderate, and nine weak. It should be noted that there was no strong positive correlation between any nutrient and productivity, even in the high-yielding population, which demonstrates that the proper balance between all nutrients is what guarantees higher yields.

Even with the split between the two populations, the most significant correlations for the complete population (Table 1) remained similar to the high-productivity population (Table 3). The productivity of the high-yielding population showed a significant and positive correlation with S, Mn and Zn. The productivity of the low-yielding population showed a significant and negative correlation with Fe (Table 2). The nutrients that showed the highest correlations with each other were Ca-Mg, Mg-S, S-Mn, within the high productivity population. On the other hand, in the low-yielding population, the highest correlations between nutrients were observed for Ca-Mg, Mg-S and Mn-Zn (Table 2).

The nutritional imbalance index (CND-r^2) and the Mahalanobis distance (D^2) in the high-yielding population were related (Fig. 2). It is observed that the higher the D2, the greater the nutritional imbalance (CND-r^2). The coefficient of determination (R^2), which represents the sample's nutritional imbalance

Table 1. Pearson correlation matrix between nutrient concentrations in leaves and vineyard productivity (t ha^{-1}) (n = 101).

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Vineyard productivity	-0.05	-0.03	0.07	0.18	0.28*	0.38*	0.11	0.21*	-0.19	0.31*	0.51*
N		0.21*	0.03	-0.22*	-0.16	0.01	0.15	-0.18	0.08	-0.01	0.00
P			0.01	0.31*	0.07	-0.03	0.04	-0.03	0.10	0.05	0.04
K				-0.02	0.00	0.40*	-0.13	0.22*	-0.04	0.29*	0.23*
Ca					0.61*	0.21*	-0.06	0.22*	0.17	0.27*	0.08
Mg						0.55*	0.12	0.44*	0.24*	0.46*	0.23*
S							0.14	0.34*	0.12	0.76*	0.54*
B								-0.03	0.16	0.13	0.02
Cu									0.16	0.15	0.31*
Fe										0.03	-0.08
Mn											0.51*

*Significant at $p < 0.05$, by the test and Tukey at 5% probability.

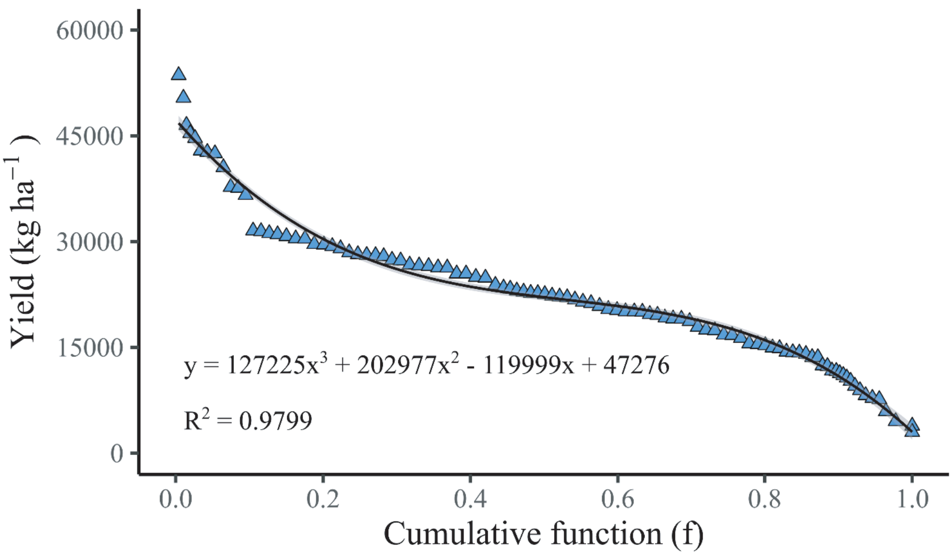

Fig. 1: Cumulative productivity function (*f*) of yield data (kg ha^{-1}) in vineyards (n = 101).

Table 2. Pearson correlation matrix between multi-nutrient variables in leaves and vineyard productivity (t ha^{-1}) (n = 101).

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Vineyard productivity	-0.32*	-0.28*	-0.31*	0.04	0.19	0.12	-0.20*	0.08	-0.45*	0.24*	0.35*
N		0.66*	0.80*	-0.29*	-0.39*	0.09	0.71*	-0.62*	0.74*	-0.55*	-0.28*
P			0.51*	0.13	-0.16	-0.21*	0.44*	-0.56*	0.53*	-0.51*	-0.24*
K				-0.35*	-0.52*	0.21*	0.55*	-0.47*	0.61*	-0.51*	-0.26*
Ca					0.53*	-0.52*	-0.30*	-0.05	-0.20*	0.01	-0.13
Mg						-0.17	-0.22*	0.05	-0.31*	0.18	-0.13
S							0.13	-0.20*	-0.01	0.19	0.10
B								-0.52*	0.62*	-0.39*	-0.35*
Cu									-0.38*	-0.01	-0.16
Fe										-0.58*	-0.44*
Mn											0.29*

* = Significant at $p < 0.05$, by the test and Tukey at 5% probability.

Table 3. Pearson correlation matrix between nutrient concentrations in leaves and productivity in high and low yield vineyards.

High yield population (n = 53)

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Productivity	0.08	0.06	0.06	0.11	0.24	0.42*	0.16	0.13	-0.13	0.39*	0.58*
N		0.29	0.06	-0.17	-0.03	0.17	0.12	-0.06	0.28*	0.33*	0.10
P			0.18	0.24	0.03	0.05	-0.22	-0.08	0.14	0.17	0.07
K				0.11	-0.02	0.25	-0.13	0.23	-0.07	0.17	0.24
Ca					0.59*	0.33*	-0.17	0.17	0.22	0.33*	0.08
Mg						0.63*	0.07	0.38*	0.23	0.49*	0.21
S							0.14	0.33*	0.27	0.75*	0.52*
B								-0.05	0.23	0.18	0.03
Cu									0.15	0.10	0.34*
Fe										0.16	-0.07
Mn											0.47*

Low yield population (n = 48)

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
Vineyard productivity	-0.05	-0.17	0.04	-0.01	-0.10	-0.02	-0.05	-0.09	-0.27	0.01	0.16
N		0.14	0.02	-0.24	-0.28*	-0.08	0.18	-0.45*	-0.06	-0.29*	-0.10
P			-0.13	0.39*	0.14	-0.11	0.20	0.06	0.07	-0.06	0.02
K				-0,17	-0,01	0,56*	-0,15	0,26	0.00	0.41*	0.24
Ca					0.61*	-0.04	0.00	0.30*	0.18	0.13	-0.05
Mg						0.36*	0.14	0.54*	0.35*	0.37*	0.10
S							0.11	0.30*	0.07	0.76*	0.56*
B								-0.06	0.14	0.07	-0.06
Cu									0.37*	0.21	-0.08
Fe										-0.03	-0.06
Mn											0.61*

* = significant at $p < 0.05$, according to Tukey test.

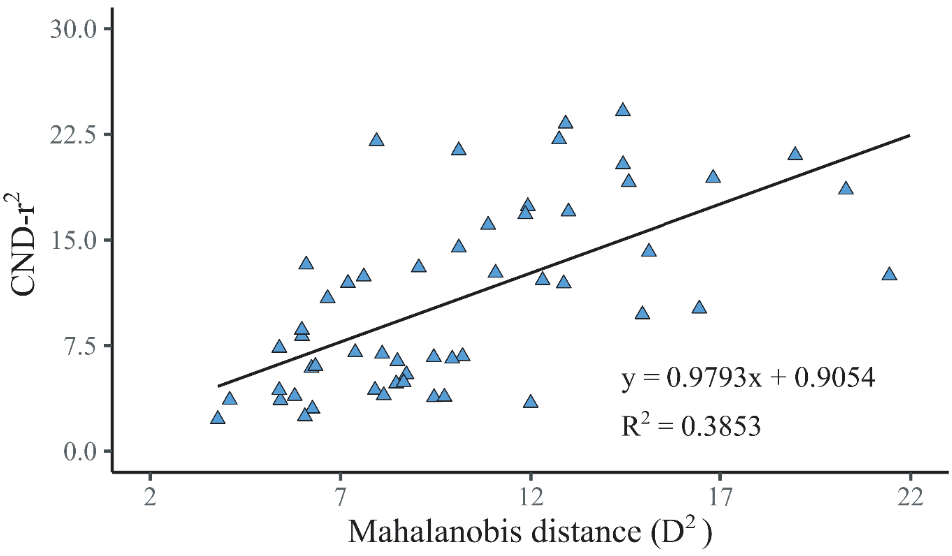

Fig. 2: Nutritional imbalance index (CND-r2) and the Mahalanobis distance (D2) of the high-yielding population (n = 53) of grape productivity.

index of the relationship between grape productivity and the CND-r^2 index was 0.40.

With the standard of the CND indexes established, the mean of the range considered normal for the chemical composition of the complete leaves of vines proposed by the recommendation of regional fertilization for the vine culture was compared (Fig. 3). The result of the CND-r^2, which is the nutritional imbalance index, was 79.20. Based on the standards established by the CND method, the concentrations proposed in the regional recommendation for the contents of K, Ca, Mg and S were overestimated.

Table 4 presents the relationship of the CND-r^2 index of all stands with productivity which, according to the linear equation, presented a coefficient of determination close to zero (R^2 = 0.05) classified as a weak correlation. Through the CND method, the CND indexes for each nutrient were calculated, which were related to their respective nutrient content determined in the leaves. The mathematical models elaborated by the regression analysis for the nutrients Ca, Cu, Mn and Zn (Table 4), obtained coefficients of determination above 80% (R^2 > 0.80), corresponding to a strong Pearson correlation.

The CL and SR, that correspond to the adequate levels for each nutrient in the plant tissue (Table 4), were obtained by equating the indexes of each equation to zero (Table 5). The SRs adequate for the evaluation of each nutrient in grapevine leaves were different from those observed in other studies carried out in Brazil (Table 5). The application of the CND method in the grapevine database showed lower SRs for macronutrients N, K, Ca, Mg and S, and the breadth of the nutritional range for nutrients N, K, Mg and Fe was smaller than the reported in the literature.

Discussion

The correlation between nutrients means that the change of one nutrient in leaves and that of the other nutrient changes proportionally (Parent *et al.*, 2013, Rozane *et al.* 2015). This finding demonstrates that analysis methods based on univariate correlations are inefficient to explain productivity, corroborating what was observed in the present study (Table 1).

The use of the multi-nutrient relationships (Parent and Dafir 1992) confers a greater number of significant correlations between the nutrient indexes and with productivity (Table 2) in

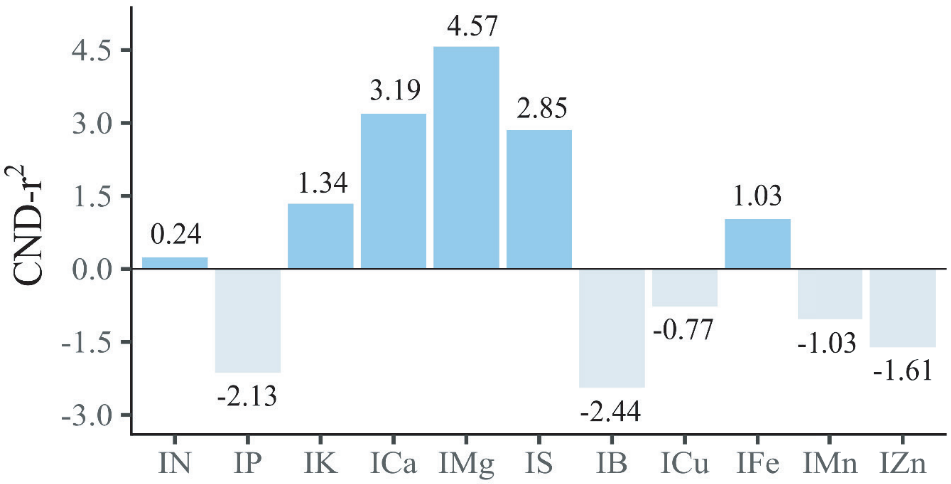

Fig. 3: Comparison between the recommended mean concentrations of nutrients in leaves for vines (Brunetto et al. 2016b) and the nutritional imbalance index (CND-r2) of the high-yielding population of grape productivity.

Table 4. Patterns of the diagnosis of nutritional composition (CND) of the reference population.

	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	
Mean	2.65	1.38	1.82	1.26	-0.21	0.17	-2.51	-3.96	-2.82	-1.51	-2.60	
Standard deviation	0.32	0.33	0.27	0.48	0.29	0.25	0.34	1.12	0.30	0.68	0.64	
	IN	**IP**	**IK**	**ICa**	**IMg**	**IS**	**IB**	**ICu**	**IFe**	**IMn**	**IZn**	**CND-$r^{2\dagger}$**
Maximum limit	2.18	2.30	2.39	1.66	2.60	2.74	1.97	2.22	1.89	1.73	2.54	23.70
Minimum limit	-1.76	-1.77	-2.13	-1.78	-2.11	-2.95	-2.26	-1.41	-1.92	-2.25	-2.34	2.47
Mean deviation	0.82	0.82	0.83	0.86	0.76	0.74	0.80	0.84	0.86	0.84	0.80	5.59
Distortion	0.38	0.23	0.20	-0.21	0.40	-0.13	0.34	0.78	0.17	-0.36	0.10	0.50

† CND-r^2: nutritional imbalance index.

relation to the use of the absolute content (Table 1), attributing an increase of 84% to the correlations between the indexes and 40% between the indexes and productivity. Oliveira *et al.* (2020) reported higher correlations between the multinutrient indexes among themselves and with productivity in relation to the absolute content in guava trees. It is worth noting that the quality of the significant relationships also increased with three strong (K-N; B-N; Fe-N) and 17 moderate (Fe-productivity; K-P; B-P; Cu-P; Fe-P; Mn-P; Mg-K; B-K; Cu-K; Fe-K; Mn-K; Ca-Mg; S-Mg; Cu-B; Fe-B; Mn-Fe; Zn-Fe) (Table 2).

It is highlighted that the results showed that productivity is dependent not only on the concentrations of a particular nutrient inside the plant, diagnosed by leaf analysis, but, especially, it is determined by the relationships between nutrients (Marschner 2012, Rozane *et al.* 2016).

The interpretation of the mean nutrient contents in the leaves of high and low yield populations were very similar in the classification as insufficient, normal or excessive (Brunetto *et al.* 2016), only differing in the Mn interpretation. The mean of nutrient concentrations in the populations evaluated, both high and low yield, did not explain the productivity of the vineyards and did not allow the indication of ranges of adequate nutritional concentrations. Thus, the results again reinforce the need to use bi- or multivariate methods to diagnose the nutritional status of plants, as they allow evaluating the interaction between each nutrient and the balance between them (Rozane *et al.* 2020, Lima Neto *et al.* 2022).

The use of the Mahalanobis distance (Fig. 2), proposed by Parent and Dafir (1992), allows the identification and exclusion of observations with imbalances, a procedure that is not possible using bivariate methods such as DRIS (Rozane *et al.* 2013). In another study with grapevine culture, where the CND method was used, the observed CND-r^2 was 0.42 (Rozane *et al.* 2016). In studies with annual cycle crops such as carrots, values of 0.34 have already been reported (Parent *et al.* 1994) and 0.32 have been reported in the potato crop (Khiari *et al.* 2001b). Higher values of determination coefficients in perennial crops, such as in grapevines, can be attributed to nutrient reserves in perennial organs, as in roots, branches older than one year, and even in the stem (Trapp *et al.* 2022). Nutrients present in perennial organs can be redistributed to annual organs in vegetative and productive periods (Paula *et al.* 2021). Thus, the accumulation and redistribution of nutrients can contribute to greater nutritional stability in fruit trees, in relation to annual cycle plants (Paula *et al.* 2021, Trapp *et al.* 2022).

Table 5. Statistical models used to find critical nutrient levels among diagnostic indexes of nutritional composition (CND).

Nutrients	Models	R^2	Critical level (CL)	Sufficiency range (SR)
N	$-0.0000x^2 + 0.0008x - 11.3320$	0.24	22.1 g kg^{-1}	19.9 – 24.0
P	$0.0000x^2 - 0.0004x + 0.1453$	0.44	6.7 g kg^{-1}	5.5-7.8
K	$-0.0000x^2 + 0.0007x - 4.3270$	0.06	9.0 g kg^{-1}	7.8-10.1
Ca	$-0.0000x^2 + 0.0005x - 2.7811$	0.81	6.5 g kg^{-1}	4.2-8.6
Mg	$1.9596\ln(x) - 14.3450$	0.70	1.5 g kg^{-1}	1.0-2.0
S	$-0.0000x^2 + 0.0024x - 3.4363$	0.41	2.0 g kg^{-1}	1.5-2.5
B	$0.0195x - 2.5929$	0.35	133.8 mg kg^{-1}	111.3-151.4
Cu	$0.7072\ln(x) - 2.5245$	0.97	36.9 mg kg^{-1}	36.9-137.1
Fe	$0.0204x - 1.8234$	0.19	87.0 mg kg^{-1}	71.7-101.8
Mn	$1.0476\ln(x) - 6.2949$	0.94	411.1 mg kg^{-1}	137.3-685.5
Zn	$1.3191\ln(x) - 6.4490$	0.88	141.8 mg kg^{-1}	51.5-237.2
Vineyard productivity	$1.3191\ln(x) - 6.4490$	0.05	-	-

Table 6. Nutrient sufficiency ranges (SR) considered adequate in grapevine leaf samples obtained by the CND method in the present study, in comparison with recommendations established by other authors for the same crop grown in Brazil.

References	N	P	K	Ca	Mg	S
			g kg^{-1}			
Rozane *et al.* (2020)	24-30	2.9-3.8	11-14	12-16	2.6-3.3	3.1-3.8
Brunetto *et al.* (2016)	16–24	1.2-4.0	8-16	16-24	2.0-6.0	-
Quaggio and Raij (1997)	30–35	2.4-2.9	15-20	13-18	4.8-5.3	3.3-3.8
Melo *et al.* (2017)	24-30	2.9-3.9	11-14	12-16	2.6-3.3	3.1-3.8
References	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**-**
			mg kg^{-1}			
Rozane *et al.* (2020)	27–41	10-14	91-142	398-586	148-254	-
Brunetto *et al.* (2016)	30–65	-	60-150	30-300	25-60	-
Quaggio and Raij (1997)	45–53	18-22	97-105	67-73	30-35	-
Melo *et al.* (2017)	26–39	10-14	89-140	390-578	150-256	-

Using the CND method, the standards of the CND-r^2 index (Table 4, Fig. 3) for each nutrient in the grapevine crop were established, based on the mean of the concentrations of each nutrient in the leaf and the standard deviation of the high-yielding population. The data shown did not provide sufficient information to explain whether grape productivity is associated with the nutritional indexes calculated by the method. We believe that the low correlation indexes are due to other factors influencing productivity that are not related to plant nutrition. In vineyards, climatic variables (e.g. temperature, precipitation and insolation) can positively or negatively affect productivity (Pio *et al.* 2018; Chen *et al.* 2020; Stefanello *et al.* 2021). But also management practices with vine pruning, fungicide and insecticide applications impact productivity (Rozane *et al.* 2015, Rozane *et al.* 2020).

The results of the CND-r^2 index (Table 4, Fig. 3) show that the data did not provide sufficient information to explain whether grape productivity is associated with the nutritional indexes calculated by the method. We believe that the low correlation indexes are due to other factors influencing productivity that are not related to plant nutrition. In vineyards, climatic variables (e.g. temperature, precipitation and insolation) can positively or negatively affect productivity (Stefanello *et al.* 2021; Rozane *et al.* 2020). But also management practices with vine pruning, fungicide and insecticide applications impact productivity (Rozane *et al.* 2015, Rozane *et al.* 2020).

Based on the standards established by the CND method (Table 4), the concentrations proposed in the regional recommendation for the contents of K, Ca, Mg and S were overestimated (Table 5). This shows that the regional recommendation for the grapevine is overestimating the need for nutrient application, which can increase their levels in vineyard soils. The contents may be above the demand of the grapevines, which can enhance transfers by leaching, but especially by surface runoff, since the vineyards are located in undulating reliefs (Stefanello *et al.* 2022). On the other hand, the contents of N, P, B, Fe and Zn were underestimated, indicating that if the regional recommendation for the grapevine was followed, the plants could present nutritional deficiency, which could even harm the productivity, quality and composition of the grape (Stefanello *et al.* 2021, Ciotta *et al.* 2021).

The results obtained by the CND method (Table 5) demonstrate that the current regional system of interpretation of nutrient concentrations in grapevine leaves promotes nutritional imbalance. Furthermore, it is clear that only one standard of recommendation for grapevines grown in two states of Brazil, such as Rio Grande do Sul (RS) and Santa Catarina (SC) (Brunetto *et al.* 2006) is unsatisfactory, reinforcing the need for generating leaf reference values that allow for regional interpretation (Rozane *et al.* 2015; Paula *et al.* 2021).

With the construction of the CND indexes (Fig. 3) from the database of vines, viticulturists will be able to adequately estimate the real need for nutrient application in vineyards (Rozane *et al.* 2020; Lima Neto, *et al.* 2022). With this, it will be possible to avoid excessive applications of nutrients in vineyards, which can raise the levels in soils above the amount needed by plants (Parent *et al.* 2013; Silva *et al.* 2021), decreasing the potential for soil and water contamination (Parent *et al.* 2013). It may also decrease the cost of fertilizer acquisition, which will increase profitability.

The SF proposed to carry out the nutritional diagnosis from the nutrient contents in leaves were compared to the indicated ranges for vines cultivated in the State of Rio Grande do Sul (RS) (Melo 2018; Brunetto *et al.* 2016, Rozane *et al.* 2020), where the present study was carried out (Table 5). They were also compared to the results observed by Quaggio and Raij (1997), in the State of São Paulo, Brazil. The greatest divergences between the appropriate leaf nutrient standards recommended in the manual that guides the largest grape producing state (Brunetto *et al.* 2016) and the present study were observed in P, Ca, Mg, B, Mn and Zn. The great divergence in the FS of Mn and Zn is due to fungicide applications carried out to control and prevent diseases in leaves and clusters, as well as it occurs in numerous other fruit trees, such as citrus (Rozane *et al.* 2015). Serra *et al.* (2010), considered the smaller amplitude of the sufficiency range as positive information, as it allows for greater precision in evaluating the results of leaf content.

Conclusions

The CND-r^2 indexes were effective in establishing the nutritional status of ' White Moscato' and 'Bordeaux' grapevines, in relation to the concentration of nutrients in N, P, K, Ca, Mg, B, Cu, Fe, Mn and Zn in leaves in deficient, adequate and excessive concentrations.

The CND methodology established the CL and SR of adequate nutrients in leaves, contributing to the establishment of the real need for nutrient application in vineyards.

Multi-nutrient combinations were more effective than the analysis of a single nutrient in expressing that the limitation of a certain element can reduce the productivity of the vines.

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Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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