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Leaf blade and petiole nutritional diagnosis for *Vitis vinifera* L. cv. 'Tempranillo' by deviation from optimum percentage method

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Abstract

Deviation from optimum percentage (DOP) is a diagnosis methodology for leaf analyses which expresses the deviation for each element with respect to its optimal concentration. This deviation is an individual index for each nutrient and allows the sorting of all the analyzed nutrients according to their limitations. A nutritional survey was undertaken over eleven years in La Rioja (Spain), to establish reference concentrations for the nutritional diagnosis of *Vitis vinifera* L., cv. 'Tempranillo' grafted on Richter-110. Reference concentrations for DOP methodology are proposed, and sensibility for the nutritional diagnosis was evaluated for blade and petiole analysis of N, P, K, Ca, Mg, Fe, Mn, Zn, Cu, and B at flowering and veraison phenological stages by comparison between DOP and sufficiency ranges (SR) methods. Results suggest that petiole has lower sensibility than blade to detect deficiencies or excesses of N, P, K, Ca, Mg, Zn, and Mn at veraison. In addition, petiole is a better tissue than blade to detect Fe and B deficiencies or excesses. Therefore, our results make possible the right choice between tissues, leaf blade or petiole, for a general nutritional diagnosis of 'Tempranillo' grapevines. On the other hand, it is possible to evaluate the status of each nutrient in each phonological stage analyzing both tissues and comparing the nutrient status to its references, DOP or SR, in the most adequate tissue.

Additional key words: nutrition; grapevines; DOP references; DOP norms.

Introduction

In general, plant diagnosis systems compare the nutrient concentration in plant tissues with respect to reference concentrations obtained from a population with optimal nutrition status, in agreement with the production objectives (yield, must/wine quality, etc.) (Walworth & Sumner, 1987; Lucena, 1997; Mourao Filho, 2004).

Several diagnosis methods are used to assess the nutritional status of a crop, including sap analysis, analyses of active metabolites (for example N-NO_3^- in petiole), or studies of specific enzymatic activities. However, mineral analysis of leaf blades and petioles are still the most widely used (Cook & Kishaba, 1956; Bonilla *et al*., 1980; Montañes *et al*., 1993; Lucena, 1997; Robinson, 2005).

Due to this, several methods for interpretation of plant analyses results have been proposed, such as sufficiency ranges (SR) method, critical values method, DRIS (diagnosis and recommendation integrated system), or DOP (deviation from optimum percentage). However, as in other crops, critical values and SR are the most widely used methodologies for nutritional diagnosis in grapevine leaf blade and petiole samples (Loue, 1990; Failla *et al*., 1995, 1997; Ciesielska *et al*., 2002; Robinson, 2005; Garcia-Escudero *et al*., 2013).

The SR method uses a comparison of the nutrient concentration with respect to different ranges of values, thus classifying each nutrient concentration as *deficient*, *low*, *adequate*, *high*, or *excessive* (Lucena, 1997; Robinson, 2005; Garcia-Escudero *et al*., 2013). On the other hand, DOP is a routine analysis

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Abbreviations used: AOC (appellation d'origine controlée); CV% (coefficient of variation percentage); DOP (deviation from optimum percentage); DRIS (diagnosis and recommendation integrated system); ICP-AES (inductively coupled plasma-atomic emission spectrometry); $mmol_c$ (millimole of charge = milliequivalent); SR (sufficiency ranges); VSP (vertical shoot position).

interpretation method which quantifies the difference between a single nutrient concentration and its reference value using a percentage expression, ranking the individual nutrient indexes according to the order of requirements, or order of limitation, from the most negative to the highest positive nutrient index (Montañes *et al*., 1993). Individual DOP indexes inform about the high-priority nutrients in a management program. Therefore, those DOP indexes can be used to estimate, also considering the concrete soil and crop growing conditions, the fertilizer amounts to be included in a fertilization program to modify the nutritional status of the crop (Montañes *et al*., 1993; Monge *et al*., 1995).

Furthermore, the sum of the absolute value of all the calculated individual DOP indexes ($\Sigma \left| DOP_i \right|$) is a general index which represents the complete nutritional balance of the plant. The $\Sigma \left| DOP_i \right|$ indicates the importance or severity of an anomalous situation.

However, DOP, as the DRIS method, is not widely used mainly due to the lack of useful references for many crops (Lucena, 1997).

Establishing norms for SR or DOP method requires extensive surveys of basic data and nutrient concentrations within a region (Sumner, 1977; Lucena, 1997). This process requires assembling a large database over time, including many sources of variation (*e.g.* climate, topography, soil test levels, etc.) which imply a reduction in the accuracy of the final norms values. However, if databases are treated correctly with appropriate statistical methods, provisional yet reliable references can be established with great economy of time and money, as opposed to references established entirely by field trials (Failla *et al*., 1993). This methodology has been successfully used to obtain references with nutritional diagnosis purposes, and even to compare between different varieties or vine growing regions (Failla *et al*., 1993, 1995; Ciesielska *et al*., 2002; Garcia-Escudero *et al*., 2013).

In this way, references generally improve their accuracy when variation sources are considered, such as genetics (Failla *et al*., 1995), variety (Christensen, 1984) and rootstock (Tardaguila *et al*., 1995), as well as growing techniques and irrigation regime. However, survey methods must assume certain variation sources, such as different soil physical-chemical properties, different environmental conditions within the region and the differences due to the effect of the agronomic year in which the samples are taken. These effects can only be minimized by limiting references to a very local scale (Failla *et al*., 1995; Robinson, 2005).

'Tempranillo' is the leading cultivar for red wine production in Spain, with more than 200,000 ha cultivated and an increasing cultivation area in other winemaking countries. The aim of this study was to establish DOP norms for leaf blades and petioles, as well as to estimate the sensitivity of the published SR norms (Garcia-Escudero *et al*., 2013), for the nutritional diagnosis of 'Tempranillo' (*Vitis vinifera* L.) grafted on Richter 110 at both flowering and veraison. Furthermore, this study uses SR, the most widely used method, and DOP to evaluate the reliability of blade and petiole tissues, at flowering and veraison, for the nutritional diagnosis of ten essential nutrients in this variety. The final objective was to improve the accuracy of grapevine nutrient diagnosis based on tissue analysis, to allow for the design of more efficient fertilization programs.

Material and methods

Survey approach to obtain the data collection

A nutritional survey was undertaken in La Rioja (north-eastern Spain) (Garcia-Escudero *et al*., 2013). Data were collected over eleven years (2000-2010) from 166 vineyards (*Vitis vinifera* L. cv. 'Tempranillo' grafted on Richter 110) at different locations throughout the AOC Rioja (1° 40' 55' to 2° 54' 46" W – 42° 4' 24" to 42° 38' 15" N). Most of the 166 analyzed vineyards were sampled over the period 2000-2002 (123 vineyards). During the 2003-2010 period, the great majority of the initially employed vineyards were discarded and 43 new vineyards were added. The total data included in the 'Tempranillo' dataset was 2,970 analyses (Table 1).

The selected vineyards represented the local variations in climatic, soil physical-chemical properties, training system, agronomic practices, yields, sampling phenological stages, and nutrient concentrations. Therefore, the dataset included data from five environmental sub-zones as well as the different soils found within the AOC Rioja.

The procedure carried out over the dataset to obtain the DOP references, as the one used previously for the determination of the SR norms (Garcia-Escudero *et al*., 2013), assumes several variation sources in the data to

Table 1. Nutrient concentration references for DOP methodology in leaf blade and petiole, at flowering and veraison, of *Vitis vinifera* L. cv. 'Tempranillo'

	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu[1]	B
	(g/100 g)					(mg kg^{-1})				
Flowering										
Blade (N = 674)	3.21	0.293	0.946	2.19	0.337	116	76.9	18.9	12.7	62.9
CV%	9.29	28.4	22.9	17.4	23.8	61.2	107	39.2	579	31.8
Petiole (N = 678)	1.01	0.318	1.55	1.49	0.609	23.6	25.5	15.8	8.9	40.7
CV%	28.9	27.2	52.3	17.8	30.3	64.6	193	52.2	96.8	16.0
Veraison										
Blade (N = 797)	2.24	0.156	0.834	3.22	0.419	149	112	17.3	160	37.1
CV%	9.11	28.3	33.8	14.8	32.4	46.0	78.6	36.6	119	30.5
Petiole (N = 791)	0.492	0.112	1.40	1.97	0.861	24.6	56.8	21.7	20.1	36.7
CV%	18.2	65.8	66.0	21.5	40.3	73.9	190	86.3	115	20.3

[1] Mean concentration with a physiological meaning cannot be determined due to fungicide residues. CV: Coefficient of variation (%) or median coefficient of variation (%).

avoid an excessive loss of information, which would otherwise lead to an overly restrictive dataset. Therefore, Gobelet, Double Cordon Royat and Guyot VSP training systems, as well as different soil management practices, such as cover crops or conventional mechanical tilling, were not distinguished when establishing the dataset to calculate both SR (Garcia-Escudero *et al*., 2013) and DOP references. Planting density ranged between 2,222 and 4,310 vines ha^{-1}. Vineyards were mainly non-irrigated and soil was mechanically tilled according to the common practices of growers in the region. In general, most of the vineyards had optimal production (higher than 3,000 kg ha^{-1}) and grape quality was within the usual values for the AOC Rioja.

Soil properties

Soil textures ranged from loam, sandy loam to clay loam soils. Soil chemical properties were: organic matter lower than 2% d.w. (Walkey-Black method); pH ranging between 6.8 and 8.5 (1:5 soil:water, 25°C); total carbonates ranging between 0.0 and 54.7% d.w. (Bernard calcimeter method); active $CaCO_3$ ranging between 0 and 14.6% d.w. (Drouineau method); electrical conductivity lower than 2.2 mS cm^{-1} (1:5 soil:water, 25°C); and cationic exchange capacity (extraction by 1M NaAc and Na determination by flame emission spectrometry) ranging between 36 and 177 mmol$_c$ kg^{-1}.

Leaf sampling

Within each vineyard, a homogeneous sampling subplot of 450 vines was selected and leaf blades and petioles were collected twice per growing season, at flowering and veraison. Thirty leaves from different sunlight exposure over the canopy were randomly collected within each subplot. One complete leaf per plant was taken from a fruit-bearing shoot of average vigour. At flowering leaves opposite to the first bunch were chosen and at veraison leaves opposite to the second bunch were selected due to the sensitivity of the cv. 'Tempranillo' to water stress. This variety is prone to an early aging of basal leaves and, therefore, the leaf opposite to the first bunch could be inadequate for the purpose of plant nutritional evaluation at the beginning of veraison (Romero *et al*., 2010).

Sample preparation and mineral analysis

Leaf blades and petioles were separated, washed with tap water, rinsed with distilled water, dried at 70°C up to constant weight (Selecta DRYBIG; J.P. Selecta, Barcelona, Spain), and ground (sieve < 0.5 mm) with an ultra centrifugal mill (ZM1; Retsch, Haan, Germany). Two subsamples of 0.200 g were used for nutrient measurement, one for N, and one for the remaining nutrients.

Nitrogen (N-organic + N-NH_4^+) was determined by the Kjeldhal method (Horneck & Miller, 1998) after a

mineralization in 5 mL of 95% H_2SO_4 with 0.200 g of catalyst (71% K_2SO_4 + 27% $CuSO_4 \cdot 5H_2O$ + 2% Se) mixture at 370°C for 45 min. Subsequently, NH_3 was distilled, collected on 2% H_3BO_3 solution and titrated with 0.025N HCl. For chemical analysis of phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B), dry subsamples were wet digested with 3 mL of 95% H_2SO_4 and 4 mL of 30% H_2O_2 by microwave method (Hoenig *et al*., 1998) and subsequently analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) (Optima 3000DV; Perkin-Elmer, Norwalk, CT, USA). Double deionized water (Milli-Q; Milli-pore, Bedford, MA, USA) was used for all dilutions. Concentrations were expressed on a dry weight basis.

Data analysis

Prior to the analysis, the dataset was checked to eliminate anomalous data due to unhealthy vines, young vineyards less than six years old or outliers (higher or lower than ±3σ from the average value). Therefore, data from vineyards that could affect the variability due to their age or their sanitary status were ruled out. However yield and must quality were not used as discriminative factors to eliminate data before the calculation of both SR and DOP norms. This was based on the assumption that the objective was to obtain general references for the AOC Rioja as a whole and the selected vineyards had, in general, optimal yields for this region.

Data were statistically evaluated from a descriptive statistical approach. DOP references were obtained from the central value of the dataset selected for each nutrient.

The procedure began with the verification of the normal distribution for each nutrient by means of the Kolmogorov-Smirnov non-parametric test, as a prior step to study the distribution of the population as a whole, with respect to the average value and standard deviation.

When the normal distribution was skewed for a specific nutrient, a log-transformation was applied to the dataset and the DOP reference for that nutrient recalculated from the average value of the log dataset.

Finally, when the log-transformation to normal distribution did not normalize the distribution, the DOP reference value which represented the optimal status for each nutrient was calculated using the median of the population, or percentile P50.

With respect to the SR method, the reference ranges of values that characterize the different nutritional status of the dataset were delimited by means of $\mu \pm k\sigma$, where the constant k is calculated for each percentage in normal distributions with average 0 and variance 1. Population was divided into five subgroups, considering the central 20% population ($\mu \pm 0.25\sigma$) as the optimal reference level for each nutrient and 60% ($\mu \pm 0.84\sigma$) of the central population to show the populations with higher and lower nutrient contents with respect to the optimal range (Garcia-Escudero *et al*., 2013). Data analysis was performed using SPSS (version 15.0; SPSS Inc., Chicago, IL, USA).

Calculation of DOP indexes

The DOP index is defined as the percentage deviation of the concentration of a nutrient with respect to the optimum concentration. This optimum concentration is that accepted as the reference for the particular tissue analyzed at a specific phenological stage (Montañes *et al*., 1993; Lucena, 1997). The DOP index is calculated by applying the following general formula:

$$\mathrm{DOP} = [(C - C_{\mathrm{ref}}) \cdot C_{\mathrm{ref}}^{-1}] \cdot 100$$

where C is the concentration of a given nutrient in either leaf blade or petiole and C_{ref} is the optimal nutrient concentration, or reference, for the same tissue. Both concentrations are expressed as percentage of dry matter. References are generally obtained for the same conditions (phenological stage, cultivar, rootstock, position on the shoot …) from an optimal population (Montañes *et al*., 1993; Lucena, 1997).

The DOP index can be positive, zero, or negative, depending if C is higher, equal, or lower than its reference (C_{ref}) and, in general, suggesting the no employ of this nutrient in the fertilization programs (positive, or zero DOP index), or suggesting the need of application of that nutrient (negative DOP index).

Additionally, the sum of the absolute values of the nutrients' DOP indexes ($\Sigma|\mathrm{DOP}_i|$) is a general index which represents the complete nutrient balance of the plant. The $\Sigma|\mathrm{DOP}_i|$ will be near zero if the sample is near to an adequate nutritional status (Montañes *et al*., 1993; Lucena, 1997).

Table 2. DOP indexes equivalent to the SR[1] ranges for macro and micronutrients in leaf blade at flowering and veraison phenological stages for nutrition diagnosis of *Vitis vinifera* L. cv. 'Tempranillo'

Blade	Flowering			Veraison		
	Low	Optimal	High	Low	Optimal	High
Macronutrient						
N	< –7.8	–2.3-2.3	> 7.8	< –7.4	–2.3-2.3	> 8.0
P	< –17.0	–6.0-5.6	> 23.3	< –14.1	–5.1-4.3	> 17.4
K	< –17.6	–5.6-5.9	> 21.4	< –24.5	–8.1-8.8	> 35.6
Ca	< –13.5	–4.2-4.4	> 15.5	< –12.5	–3.7-3.7	> 12.5
Mg	< –16.9	–6.4-5.8	> 23.1	< –25	–8.4-8.5	> 33.0
$\Sigma\lvert DOP_i\rvert$		–24.4-24.0			–27.6-27.6	
Micronutrient						
Fe	< –24.8	–9.9-12.6	> 54.3	< –33.2	–10.2-10.2	> 38.1
Mn	< –30.1	–11.3-13.2	> 44.7	< –31.9	–11.8-10.3	> 39.2
Zn	< –19.9	–7.5-7.4	> 30.1	< –19.4	–5.8-7.0	> 30.1
Cu[2]	< –29.2	–8.7-30.2	> 544	< –63.1	–27.0-38.2	> 119
B	< –23.2	–8.0-6.5	> 27.8	< –20.1	–7.7-7.6	> 29.2
$\Sigma\lvert DOP_i\rvert$		–36.7-39.7			–35.5-35.1	

[1] References for sufficiency ranges method (Garcia-Escudero *et al*., 2013). [2] Ranges with a physiological meaning cannot be determined due to fungicide residues. [3] The $\Sigma|DOP_i|$ is calculated without Cu.

Results

Obtaining SR and DOP reference concentrations

Table 1 shows DOP reference values of macro and micronutrients for leaf blade and petiole tissues, at both flowering and veraison. The statistical procedure to obtain DOP references showed that a normal distribution was only observed for N data in leaf blade at flowering, Ca in leaf blade at veraison, and P in leaf petiole at flowering. The log-transformation to normal distribution was effective for K and Ca in leaf blade at flowering; N in blade at veraison; Ca and Mg in leaf petiole at flowering; and Mg in petiole at veraison. Finally, the DOP reference value, which represents the optimal status for the rest of macronutrients and for all micronutrients, was calculated using the median of the population, or percentile P50.

The coefficient of variation percentage (CV%) for each reference DOP index is also shown in Table 1. The CV% was higher for petiole than for blade for N, K, Mg, Mn and Zn at both phenological stages, as well as for P, Ca and Fe at veraison. However, these three elements showed similar CV% for both tissues at both phenological stages. On the other hand, B showed higher CV% for blade than for petiole.

Blade *versus* petiole

The SR categories for the nutritional status of the vines (Garcia-Escudero *et al*., 2013) can be extrapolated to their corresponding DOP indexes for each nutrient using the DOP references showed in Table 1. Both SR and DOP references were calculated using the same dataset and, therefore, the calculated DOP ranges will be an estimation of the sensitivity of the nutrient ranges in the SR method.

DOP indexes for leaf blades at flowering and veraison, corresponding to the optimal range (central 20% population) in the SR methodology, ranged between ±2 for N, ±4 for Ca, ±6 for P, ±8 for K, Mg, Zn and B, and ±13 for Fe and Mn (Table 2).

On the other hand, DOP indexes for petioles, which limit the SR optimal range (central 20% population) also at flowering and veraison, ranged between ±8 for N, ±19 for K, ±6 for Ca, ±10 for Mg, Fe and Zn, and around ±5 for B (Table 3). In addition, differences between flowering and veraison were found, ranging

Table 3. DOP indexes equivalent to the SR[1] ranges for macro and micronutrients in leaf petiole at flowering and veraison phenological stages for nutrition diagnosis of *Vitis vinifera* L. cv. 'Tempranillo'

Petiole	Flowering			Veraison		
	Low	Optimal	High	Low	Optimal	High
Macronutrient						
N	< –24.7	–7.1-8.2	> 25.2	< –12.3	–4.2-4.0	> 14.4
P	< –22.9	–6.8-6.8	> 22.9	< –40.2	–14.6-17.4	> 61.6
K	< –37.6	–15.1-13.2	> 52.2	< –49.7	–19.1-19.6	> 68.0
Ca	< –13.6	–4.3-4.4	> 15.8	< –15.8	–6.0-5.6	> 19.8
Mg	< –22.3	–7.2-7.8	> 28.7	< –29.0	–9.7-10.7	> 40.8
$\Sigma\lvert DOP_i\rvert$		–40.5-40.4			–53.6-57.3	
Micronutrient						
Fe	< –24.6	–8.3-7.3	> 30.4	< –21.1	–7.4-9.8	> 33.3
Mn	< –32.1	–10.0-13.0	> 49.3	< –58.1	–21.9-30.8	> 146
Zn	< –34.1	–10.6-10.6	> 45.9	< –31.2	–10.3-10.2	> 38.7
Cu[2]	< –24.9	–6.5-15.0	> 73.5	< –55.6	–22.6-29.2	> 128
B	< –10.9	–2.9-3.6	> 13.3	< –13.6	–3.7-4.8	> 19.6
$\Sigma\lvert DOP_i\rvert$[3]		–31.8-34.5			–43.3-55.6	

[1] References for sufficiency ranges method (Garcia-Escudero *et al*., 2013). [2] Ranges with a physiological meaning cannot be determined due to fungicide residues. [3] The $\Sigma\lvert DOP_i\rvert$ is calculated without Cu.

from ±7 at flowering and ±17 at veraison for P (Table 3), and from ±13 at flowering and ±30 at veraison in the case of Mn. Finally, sometimes DOP indexes for Cu showed a range higher than 30 due to the high variability linked to phytosanitary applications with Cu based products (Table 3).

General DOP indexes: $\Sigma\lvert DOP_i\rvert$

The $\Sigma\lvert DOP_i\rvert$ index, obtained from the sum of the individual DOP indexes calculated for the macronutrients (N, P, K, Ca, and Mg) and micronutrients (Fe, Mn, Zn, and B) optimal ranges for the SR method are shown separately for leaf blade and petiole (Tables 2 and 3). The $\Sigma\lvert DOP_i\rvert$ ranges around ±24 at flowering and ±28 at veraison for leaf blade (Table 2). For petiole it ranges around ±40 at flowering and between –54 and +57 at veraison (Table 3). With respect to micronutrients, the $\Sigma\lvert DOP_i\rvert$ index calculated from the Fe, Mn, Zn, and B DOP indexes ranged between –37 and +40 at flowering and ±35 at veraison for blade, and between –32 and +35 at flowering and between –43 and +56 at veraison for petiole (Tables 2 and 3).

Discussion

DOP reference concentrations

The DOP references are the mean values or center values which represent the dataset as a whole. Concentrations below or above of the average value, or reference value, for each nutrient, produce negative or positive DOP indexes, respectively, and therefore a corrective fertilization plan or a reduction of the nutrient in the fertilization plans must be considered. Finally, references will be less reliable when the regional conditions of the studied vineyard are different to the ones where references were originally obtained (Failla *et al*., 1995; Robinson, 2005) or if references are employed for the nutritional diagnosis of other varieties.

In this sense, there are some differences when DOP norms for 'Tempranillo' are compared to mean values (or mean values calculated from a reference range) obtained in other viticultural areas or for other varieties. With respect to Australian references, 'Tempranillo's DOP norms for leaf blade at flowering showed lower values for all nutrients, with the exception of Ca (Robinson, 2005). 'Barbera' and

'Nebbiolo' varieties present references with lower concentrations for N, P and B, and higher values for Ca, Fe, Mn, and Zn (Ciesielska *et al*., 2002). Furthermore, 'Tempranillo' DOP norms showed higher P, Ca, and Mg concentrations, and lower N and K, with respect to Bordeaux values (Loue, 1990). However, when were compared with Italian reference concentrations, 'Tempranillo's DOP norms showed lower concentration for all nutrients except for N, P, Mg, and B (Failla *et al*., 1993).

The DOP norms for leaf blade at veraison showed lower values for N, P, K, Zn, and B, and only a higher concentration in the case of Ca when compared to Australian references (Robinson, 2005). However, Bordeaux references showed lower concentration for N, Ca, and Mg, and higher values for P, and K (Loue, 1990). The same was observed for Italian references, which also showed higher values at veraison for Fe, Mn, Zn, and Cu, and lower values only for B (Failla *et al*., 1993).

With respect to leaf petiole at flowering and veraison, Bordeaux references (Loue, 1990) and 'Cabernet Sauvignon' references (Fraguas *et al*., 2003) showed higher values than 'Tempranillo's DOP norms, with the exception of Mg. Australian references also show a similar pattern for petiole at flowering (Robinson, 2005).

These comparisons set the importance of establishing references for each variety-rootstock combination. Moreover the influence of local conditions such as different soils, weather, and vineyard management must also be taken into account.

Finally, the use of Cu-based products in vineyards for phytosanitary purposes prompts adsorption processes of Cu by the leaf surface. This adsorption increased the total Cu concentration analyzed and it avoided obtaining a Cu reference with a real physiological meaning.

DOP *versus* SR methodology

The SR methodology classifies the nutrient concentration within different concentration ranges to assess the nutritional status of the crop (Lucena, 1997; Robinson, 2005; Garcia-Escudero *et al*., 2013). Therefore, this method does not strictly quantify the differences with respect to the norms.

The SR references used for the 'Tempranillo' variety are divided in subgroups that classify nutrient concentration as *low*, *lower than optimal*, *adequate*, *higher than optimal*, or *high*. For this classification the central 20% population ($\mu \pm 0.25\sigma$ or percentile P40 and P60) was considered (Garcia-Escudero *et al*., 2013) the optimal reference range and thus a sample within these limits is classified as *adequate*; the 60% ($\mu \pm 0.84\sigma$ or percentile P20 and P80) of the central population were classified as *higher or lower than optimal* nutrient concentration respectively; and finally, the population below or above $\mu \pm 0.84\sigma$ was classified as *low* or *high* concentration respectively (Garcia-Escudero *et al*., 2013).

The SR adequate range shows a range of DOP indexes for each nutrient within which a difference from zero would be irrelevant according to the SR diagnosis criteria. However, DOP methodology will be more sensitive within the SR adequate range, showing low positive or negative indexes (Tables 2 and 3). Therefore, besides the advantage of ranking the analyzed nutrients with respect to their order of limitation, DOP can detect slight deficiencies, which is not possible by using the SR methodology. For the rest of the SR nutritional categories, the DOP indexes sign are in accordance with the category status defined by the SR methodology.

Blade *versus* petiole

The ranges of DOP indexes, calculated from the SR adequate ranges, allow the estimation of blade and petiole sensibility for nutritional diagnosis of 'Tempranillo'.

The general DOP index, $\Sigma|DOP_i|$, obtained from the sum of the individual DOP indexes calculated for the macronutrients (N, P, K, Ca, and Mg) and micronutrients (Fe, Mn, Zn, and B) optimal ranges in the SR method could be a measurement of the general sensibility of those SR references in both analyzed tissues.

The $\Sigma|DOP_i|$ for macronutrients (Tables 2 and 3) show that petiole's $\Sigma|DOP_i|$ range almost doubles the one from blade at both flowering and veraison, suggesting that blade will be a better tissue for nutritional diagnosis of this variety.

However, for an accurate evaluation of the nutritional status of the plant it is better to evaluate each nutrient individually using the most representative tissue for it. Individual DOP ranges confirm, in general, that all macronutrients analyzed

showed smaller DOP ranges for blade than for petiole at both flowering and veraison (Tables 2 and 3). This behaviour is caused by the relatively broader optimal ranges for petiole when comparing them to blade ranges in the SR method (Garcia-Escudero *et al*., 2013). Therefore, individual DOP ranges also suggest that petiole had lower sensitivity to detect individual deficiencies or excesses of any macronutrients.

However, the correct procedure to determine which tissue is better for nutritional diagnosis needs specific studies to evaluate the variations of the nutrient concentrations in both tissues throughout the crop season, as well as the variability within each sampling moment (Benito *et al*., 2013; Romero *et al*., 2013). In a previous two-year nutritional monitoring study throughout the crop season for 'Tempranillo' variety (Romero *et al*., 2013), similar results for macronutrients were found. Only P and Mg differed, showing similar variability in blade and petiole at flowering. Furthermore, Benito *et al*. (2013), in a four-year monitoring study with 'Garnacha Tinta' ('Red Grenache') variety, found similar results for N, P, Ca, and K than those obtained from the DOP indexes, while Mg differed, showing lower variability for petiole throughout the season.

Therefore, variability in the nutrient concentration of a tissue can be high even within the same vineyard (Benito *et al*., 2013; Romero *et al*., 2013); and therefore this behaviour will be reflected when a dataset is collected for establishing reference values through nutritional surveys. This might lead to the establishment of excessively broad reference levels which would impede an accurate diagnosis that is in accordance with the similarity between the results from the survey and from the monitoring studies. In this sense, the CV% for the reference DOP indexes (Table 1), showed lower CV% in blade for N, K, and Mg at both phenological stages. Furthermore, blade also showed lower CV% for P and Ca at veraison, while their CV% was similar for both tissues at flowering. Therefore, the width of DOP indexes for the optimal ranges also suggested similar conclusions as CV% (Tables 1 to 3).

With respect to micronutrients, $\Sigma|DOP_i|$ shows similar ranges for blade and petiole at flowering, while petiole shows a broader range than blade at veraison. This general result suggests that petiole also showed lower sensibility than blade to detect deficiencies or excesses of micronutrients at veraison.

Individual DOP ranges for each micronutrient showed that Fe and B had a closer DOP range in leaf petiole with respect to blade at both flowering and veraison; while Zn at both phenological stages and Mn at veraison, showed a closer DOP range in leaf blade (Tables 2 and 3). Therefore, petiole will be a better tissue to nutritional diagnosis of Fe and B, leaf blade will be a better tissue for Zn, while Mn diagnosis showed a similar accuracy in both tissues. In this sense, DOP references for Mn and Zn in blade, and B in petiole, showed lower CV% at both phenological stages (Table 1).

In general, similar results as DOP ranges were found for Mn at flowering, Zn, and B, in a monitoring study (Romero *et al*., 2013) for 'Tempranillo' variety. However, Mn at veraison, and Fe at any phenological stage had similar variability in both tissues (Romero *et al*., 2013). On the other hand, similar results as DOP ranges were found for 'Garnacha Tinta' variety, with the exception of Mn at flowering (Benito *et al*., 2013).

Therefore, the width of the DOP ranges suggested the same conclusions for 'Tempranillo' as Romero *et al*. (2013) for Zn, B, and Mn at flowering. Furthermore, DOP ranges suggested, for those elements which had similar variability according to the works of Romero *et al*. (2013) (Mn at veraison, and Fe at both phenological stages), that leaf blade could be a better estimator for Mn while leaf petiole will be a better tissue for nutritional diagnosis of Fe (Tables 2 and 3).

In summary, reference concentrations to nutritional diagnosis of leaf blade and petiole tissues of *Vitis vinifera* L. cv. 'Tempranillo' grafted on Ritchter-110, at flowering and veraison, have been proposed for the DOP method.

The CV% of DOP references and the calculation of the DOP indexes for the SR optimal ranges suggest that blade has higher sensibility than petiole to detect deficiencies or excesses of N, P, K, Ca, Mg, and Zn, at both flowering and veraison stages in the SR method, as well as for Mn at veraison. On the other hand, petiole shows higher sensibility than blade to detect Fe and B deficiencies or excesses in leaf analysis. Therefore, a technician can choose the best option for its interest: choosing to diagnostic the nutritional status of the vineyard using a single analysis of leaf blade or petiole, or analyze both tissues and evaluating each nutrient in the more appropriate tissue, and of course using the corresponding reference, DOP or SR in this case, for the phenological stage of the sampling.

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