SUFFICIENCY RANGES (SR) AND DEVIATION FROM OPTIMUM PERCENTAGE (DOP) REFERENCES FOR LEAF BLADE AND PETIOLE ANALYSIS IN 'RED GRENACHE' GRAPEVINES

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Abstract

Aim: To obtain specific references for the nutritional diagnosis of ten essential nutrients for leaf blade and petiole of 'red Grenache' (*Vitis vinifera* L.).

Methods and results: Leaf blades and petioles from 36 vineyards of 'red Grenache' (*Vitis vinifera* L.) grafted on Richter 110 were collected and analyzed at flowering and veraison between 1992 and 2008. Using the compiled data bank, nutritional references for ten elements (N, P, K, Ca, Mg, Fe, Mn, Zn, Cu and B were calculated. Optimal values were those around the central data ($\mu \pm 0.25\sigma$), while excessive and deficient values were those beyond the tails of the distribution ($\mu \pm 0.84\sigma$). Percentile calculation was performed when transformations to normal distributions became unlikely.

Conclusion: References for Sufficiency Ranges (SR) and Deviation from Optimum Percentage (DOP) methods were obtained for those ten nutrients studied.

Significance and impact of the study: The proposed 'red Grenache' references for leaf blade and petiole contribute to the improvement of the accuracy of 'red Grenache' grapevine nutrient diagnosis based on tissue analysis. These references are a guide to assess the nutritional status of 'red Grenache' grapevine around the world in general and, with higher accuracy, for the Rioja region and areas with similar vineyard conditions.

Key words: leaf analysis, DOP references, *Vitis vinifera* L., Grenache noir (Garnacha tinta)

Résumé

Objectif: Obtenir des références spécifiques pour le diagnostic nutritionnel de dix éléments nutritifs essentiels au limbe et pétiole de 'Grenache noir' (*Vitis vinifera* L.).

Méthodes et résultats: Les limbes et pétioles des feuilles provenant de 36 vignobles de 'Grenache noir' (*Vitis vinifera* L.) greffés sur Richter 110 ont été recueillis et analysés à la floraison et à la véraison entre 1992 et 2008. À partir de la banque de données, des références nutritionnelles pour dix éléments (N, P, K, Ca, Mg, Fe, Mn, Zn, Cu et B) ont été calculées. Les valeurs optimales étaient celles autour de la valeur centrale de données ($\mu \pm 0.25\sigma$), tandis que les valeurs excessives et déficientes étaient celles au-delà des extrémités de la distribution ($\mu \pm 0.84\sigma$). Lorsque les valeurs n'étaient pas conformes à une distribution normale et que la transformation logarithmique n'était pas efficace, les percentiles correspondants ont été calculés.

Conclusion: Les références pour les méthodes Gammes de Suffisance et Déviation du Pourcentage Optimal ont été obtenues pour les dix nutriments étudiés.

Signification et impact de l'étude: Les références pour le limbe et le pétiole de 'Grenache noir' contribuent à l'amélioration du diagnostic de nutriments. Ces références sont un guide pour évaluer l'état nutritionnel des vignes de 'Grenache noir' dans le monde et, avec une plus grande précision, dans la région de Rioja et dans d'autres régions similaires.

Mots clés: analyse foliaire, références DOP, *Vitis vinifera* L., Grenache noir (Garnacha tinta)

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INTRODUCTION

Vineyard fertilization has been and still remains a common cultural practice carried out to achieve different objectives mainly related to grape quality. However, nutritional imbalances often occur together with a loss in must quality. For instance, the excessive intake of potassium promotes a loss of acidity which results in reduced colour stability and poor taste (Kodur, 2011). Grape quality and agronomic practices that respect the environment are top priorities in current viticulture versus high yield criteria. Yield and quality are closely linked to the nutritional status of the crop (Champagnol, 1990); however, the nutritional requirements leading to a quality vintage are not yet fully established and therefore continue to be a matter of great interest and ongoing research. In this sense, both plant tissue and soil analyses have been widely used to characterize the nutritional status of the vineyard (Kliewer, 1991; Robinson, 2005), and leaf analyses are widely recognized as the most reliable method of determining the nutritional status of grapevines (Lucena, 1997).

The most common methods for nutritional diagnosis of leaf tissues are the Critical Values and the Sufficiency Ranges (SR) methods. Both methods use the individual total nutrient concentrations in dry tissues separately, comparing them to reference values obtained from optimal populations (Sumner, 1978; Lucena, 1997).

The Deviation from Optimum Percentage (DOP) method is a routine analysis interpretation method which compares nutrient concentrations to the references using a percentage expression (Montañés *et al*., 1993; Lucena, 1997). This method quantifies the difference between a single nutrient concentration and its reference value, offering the advantage of ranking the order of requirements or order of limitation from the most negative to the highest positive nutrient index (Montañés *et al*., 1993). DOP indexes help us to determine which nutrients need to be included in a fertilization program (Montañés *et al*., 1993; Monge *et al*., 1995).

Furthermore, the sum of the absolute value of the different DOP indexes ($\sum|DOP_i|$) is a general index which represents the complete nutritional balance of the plant and indicates the importance or severity of an anomalous situation. However, the DOP method is not widely used mainly due to the lack of useful references for many crops (Lucena, 1997).

A large data bank of leaf analysis gathered over a long period of time is necessary to establish adequate and representative references with the objective of monitoring the nutritional status of a vineyard within the environmental and cultural factors of a particular area (Sumner, 1978; Failla *et al*., 1993b; Lucena, 1997; García-Escudero *et al*., 2013). Large data banks are often used to define desirable ranges for leaf analysis interpretation (Sumner, 1978; Lucena, 1997; García-Escudero *et al*., 2013) through statistical operations that explain the variability of nutrient concentrations inside the population (Sumner, 1978). As a result, different sufficiency ranges have been published for different regions and variety-rootstock combinations. Failla *et al*. (1993a) proposed one common sufficiency range using a dataset which contains data from thirteen varieties grafted on ten different rootstocks, grown at four locations in northern Italy on different soil types. Failla *et al*. (1993b) proposed reference values for several cultivars and regions in north-central Italy. Ciesielska *et al*. (2002) in the Piedmont region (Italy) developed individual standard values for leaf nutrients for two cultivars, 'Barbera' and 'Nebbiolo'. Stringari *et al*. (1997) proposed standards by developing homogeneous groups of grape varieties from different appellations from northern and central Italy. Pacheco *et al*. (2010) developed specific ranges for 'Tricandeira' grafted on Richter 99 for the Portuguese region of Borba (Alentejo). Failla *et al*. (1995) proposed standards for each of the most important varieties located in eight subzones within the Tuscany region. Considering a long-term survey in representative viticultural regions in Italy, standards have been proposed for macronutrients and trace-elements in leaf blades at fruit-set and veraison (Bavaresco *et al*., 2010). Finally, García-Escudero *et al*. (2013) proposed reference values for 'Tempranillo' in the Rioja AOC of Spain. In spite of the regional character of all these references, they are widely used for nutritional diagnosis around the world, even for different varieties.

The accuracy of the ranges obtained improves when the number of foliar analysis in the dataset increases and when variation sources such as variety and rootstock combinations, seasonal weather conditions, soil types and cultural practices (Robinson, 2005) decrease. These sources of variation in nutritional status often generate wide reference ranges (Sumner, 1978). Therefore, the most accurate reference ranges are achieved at a local scale (Failla *et al*., 1995; Robinson, 2005). Furthermore, many authors even proposed a yearly adjustment of the references to consider the impact of seasonal weather conditions over the years (Failla *et al*., 1993a).

'Red Grenache' is one of the most common wine grape varieties in the world, and the second in the Rioja region. This is an area of Spain with a strong wine tradition, well known for producing top quality wines. The aim of this study was to develop reference levels for the nutritional diagnosis of ten essential nutrients for leaf blades and petioles of 'red Grenache' grapevine grafted on Richter 110, at the flowering and veraison phenological stages. Those general reference levels will allow us to improve the accuracy of the 'red Grenache' nutritional diagnosis in order to design better fertilization programs. Also, considering that the proposed 'red Grenache' references were calculated from populations with optimal yield and grape juice quality parameters, the use of these references could contribute to the improved quality of the final wines by means of the general improvement of the nutritional status of the vineyards grown with 'red Grenache'.

MATERIALS AND METHODS

1. Sampling area and establishment of the data bank

A data bank of blade and petiole analysis was compiled over seventeen years (1992-2008) in the north-eastern area of Spain within the Rioja AOC (1º 40' 55" to 2º 54' 46" W – 42º 4' 24" to 42º 38' 15" N). A total of thirty-six representative fully productive vineyards (more than 5 years old) of *Vitis vinifera* L. cv. red Grenache (Garnacha tinta, Grenache noir) grafted on Richter 110 were selected to create a representative data bank in order to obtain reference levels for the whole region. The data bank included different descriptive parameters of each vineyard: (i) plot characteristics (year of planting, geographic coordinates, altitude, percent slope, chemical soil analysis), (ii) vineyard design (spacing, planting density, row orientation, training system), (iii) mineral nutrient concentration in leaf tissues (blade or petiole) and sampling time (flowering or veraison), (iv) plant material (variety, rootstock), (v) cultural practices (vineyard training, fertilization, hydric regime, soil management), (vi) yield, and (vii) environmental factors (pests, diseases, climatic incidents and physiological alterations). The dataset included data from five environmental subzones and the different soils found within the Rioja AOC. In all, the 'red Grenache' dataset consisted of a total of 950 blade and petiole analyses.

The vineyards chosen for this study had mainly non-irrigated and mechanically-tilled soils. Planting density ranged between 2,700 and 3,205 vines·ha^{-1}, and the training systems were Gobelet, Vertical Shoot Position (VSP) Double Cordon Royat, and VSP Guyot. In general, most of the vineyards had optimal production (higher than 3,000 kg·ha^{-1}) and the average bunch weight was 236 g. Grape quality was within the usual values for the Rioja AOC. Average total soluble solid content was 22.5 ºBrix, pH was 3.36, and titratable acidity was 6.87 g·L^{-1} expressed as tartaric acid. The average cumulative annual rainfall was 451 mm for the period 1992-2008.

2. Soil properties

Soil textures ranged between loam, sandy loam and clay loam (Soil Survey Staff, 2010), and chemical analysis showed a generally low organic matter concentration (Walkley-Black method) that ranged between 0.5 and 1.7 g·100 g^{-1} dry weight (d.w.). Cation exchange capacity (C.E.C.) (extraction by sodium acetate 1 M and Na determination by flame emission spectrometry) was within acceptable limits (5.95 and 19.3 mmol$_c$·100 g^{-1} d.w.), as well as active limestone, determined by the Drouineau method (0.20 and 12.6 g·100 g^{-1} d.w.), and total carbonates (Bernard calcimeter method), which ranged between 2.05 and 40.6 g·100 g^{-1} d.w. The pH (1:5, 25ºC) was high, between 7.90 and 8.65, while electrical conductivity (E.C.) (1:5, 25ºC) was low, between 0.08 and 0.40 mmhos·cm^{-1}. Exchangeable bases K, Ca and Mg in soil (1 M ammonium acetate extraction) were determined by flame atomic absorption (Ca and Mg) and flame emission (K) spectrometry. The concentration of bases for the soils included in the dataset was, in general, adequate for grapevine cultivation: Ca ranged between 8.2 and 22.2 mmol$_c$·100 g^{-1} d.w., Mg ranged between 0.32 and 1.53 mmol$_c$·100 g^{-1} d.w., and K ranged between 0.62 and 5.91 mmol$_c$·100 g^{-1} d.w. On the other hand, P (Olsen method) was higher than 5.38 mg·kg^{-1} d.w.

3. Leaf sampling

A homogeneous subplot of 450 vines was selected from each vineyard and leaf blades and petioles were collected twice per growing season, at flowering and veraison. Thirty leaves from different sunlight exposure were randomly collected within each subplot. One 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 an early aging of basal leaves. Therefore, the leaf opposite to the first bunch could be inadequate for plant nutritional evaluation at the beginning of veraison (Romero *et al*., 2010).

4. Sample preparation and mineral analyses

Leaf blades and petioles were separated, washed three times with tap water, rinsed with distilled water, oven-dried (Dry-Big; J.-P. Selecta, Barcelona, Spain) at 70ºC for 48 h, ground with an ultracentrifugal mill (ZM1; Retsch, Haan, Germany) to pass through a 0.50-mm mesh screen, and finally stored at room temperature to be analyzed. For chemical nutrient analysis, 0.200 g of the ground sample were used.

Nitrogen (N-organic + N-NH_4^+) was analyzed by the Kjeldahl method (Horneck and Miller, 1998) after mineralization in 5 mL H_2SO_4 with a catalyst (K_2SO_4 + $CuSO_4 \cdot 5H_2O$ + 2% Se) mixture at 370ºC for 45 min. Subsequently, NH_3 was distilled, collected in 2 % H_3BO_3 and titrated with HCl 0.025 N. For chemical analysis of the other nine nutrients, dry samples were wet-digested with 3 mL 95 % H_2SO_4 and 4 mL 30 % H_2O_2 by the microwave method (Hoenig *et al*., 1998). Phosphorus, K, Ca, Mg, Fe, Mn, Zn, Cu and B were determined by Inductively Coupled Plasma-Atomic Emission Spectrometry (Optima 3000DV; PerkinElmer, Norwalk, CT, USA). Double deionized water (Milli-Q; Millipore, Bedford, MA) was used for all dilutions. Concentrations were expressed in terms of dry weight, using g·100 g^{-1} for macronutrients and mg·kg^{-1} for micronutrients.

5. Statistical 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 references. This was based on the assumption that the objective was to obtain general references for the ‘red Grenache’ variety and the selected vineyards had, in general, optimal yields for this region.

Data were statistically evaluated from a descriptive statistical approach (modified from Stringari *et al*., 1997; García-Escudero *et al*., 2013). 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.

With respect to the SR method, the reference ranges that characterize the different nutritional status of the dataset were delimited by means of $\mu \pm k \cdot \sigma$, where the constant k is calculated for each percentage in normal distributions where the average is 0 and the 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 (García-Escudero *et al*., 2013). Furthermore, DOP references were obtained from the central value (μ) of the dataset selected for each nutrient.

When a normal distribution was not verified for a specific nutrient, data was transformed logarithmically and the SR subgroups and the DOP reference for each nutrient recalculated from the average value of the Log dataset.

Finally, when the Log-transformation to normal distribution did not normalize the distribution, the percentiles (P20, P40, P60 and P80) were calculated to obtain the SR subgroups while the DOP reference value which represented the optimal status for each nutrient was calculated using the median of the population, or percentile P50.

Data analysis was performed using SPSS (version 15.0; SPSS Inc., Chicago, IL, USA).

RESULTS

1. Comparison of the ‘red Grenache’ dataset with respect to international references

Tables 1 and 2 show the percentage of samples from the ‘red Grenache’ leaf blade dataset that are within the optimal range according to references published by other authors for different tissues, phenological stages, grapevine cultivars and regions; Tables 3 and 4 show the same analysis for petiole.

For leaf blade at flowering (Table 1), the dataset optimal percentage for N ranged from 7.8 % with respect to the Trentino references (Italy) (Failla *et al*., 1993a) to 98.8 % of the optimal values when using the Bordeaux references (Loué, 1990). Phosphorus ranged from 20.4 to 98.2 %, K from 19.8 to 100 %, Ca from 21.6 to 92.8 %, Mg from 13.2 to 97 %, Fe from 23.0 to 100 %, Mn from 12.0 to 99.4 %, Zn from 0.6 to 18.7 %, Cu from 0 to 93.8 %, and B from 2.5 to 97.5 % (Table 1).

Leaf blade at veraison (Table 2) and leaf petiole at flowering (Table 3) and veraison (Table 4) also showed strong differences between diagnosis results using international references on the ‘red Grenache’ dataset.

For leaf blade at veraison (Table 2), the dataset optimal percentage ranged between 18.5 and 98.4 % for N, 27.1 and 100 % for P, 7.4 and 93.1 % for K, 22.8 and 98.4 % for Ca, 18.5 and 100 % for Mg, 13.3 and 100 % for Fe, 23.0 and 100 % for Mn, 11.6 and 58.1 % for Zn, 1.2 and 100 % for Cu, and 11.6 and 87.8 % for B. Therefore, the biggest difference between ranges was observed for Cu between the Italian references (Bavaresco *et al*., 2010) and the Trentino references (Failla *et al*., 1993a).

With respect to petiole at flowering, N ranged from 6.6 to 97 %, P from 0 to 100 %, K from 26.3 to 100 %, Ca from 9.0 to 99.4 %, Mg from 15.0 to 100 %, Fe from 4.4 to 95.6 %, Mn from 5.2 to 70.7 %, Zn from 0 to 59.3 %, Cu from 20.8 to 98.1 %, and B from 1.7 to 100 %. In this case, the biggest difference between dataset optimal percentages was observed for P using 'Cabernet sauvignon' references from Brazil (Fráguas *et al*., 2003) and Bordeaux references (Loué, 1990).

Table 1 - Dataset percentages of nutrient concentration in leaf blade at flowering of 'red Grenache' within the optimal values for different cultivars and/or regions.

Nutrient	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	Blade at flowering									
International references	$g \cdot 100g^{-1}$ d.w.					$mg \cdot kg^{-1}$ d.w.				
Trentino†	2.20-2.70	0.15-0.25	1.10-1.50	1.90-2.70	0.20-0.34	> 45	> 25	> 25	> 4	18-32
Italy‡	2.08-2.95	0.14-0.26	0.78-1.40	1.43-2.55	0.19-0.37	65-300	50-500	20-250	10-20	20-70
North-central Italy§	2.40-3.10	0.18-0.30	0.80-1.50	2.00-3.40	0.22-0.40	80-210	75-370	26-120	> 20	24-48
Australia¶	3.00-5.00	0.25-0.40	1.00-1.80	1.20-2.80	0.30-0.60	-	30-200	35-60	10-100	30-200
cv. Tempranillo (Rioja)††	3.13-3.28	0.28-0.31	0.89-1.00	2.10-2.29	0.32-0.36	105-131	68-87	18-20	12-17	58-67
Bordeaux‡‡	2.48-3.71	0.18-0.48	0.61-1.94	0.94-2.65	0.13-0.46	-	-	-	-	-
	Dataset percentages									
Number of cases	167	167	167	167	167	161	167	155	128	157
Trentino†	7.8	42.5	19.8	63.5	53.3	100	99.4	5.8	93.8	2.5
Italy‡	37.7	49.1	88.6	80.2	68.3	93.2	95.2	18.7	27.3	63.1
North-central Italy§	60.5	68.3	88.0	61,7	68.9	72.0	80.8	3.9	0.0	8.3
Australia¶	57.5	52.1	40.7	92.8	53.9	-	97.0	0.6	27.3	97.5
cv. Tempranillo (Rioja)††	16.2	20.4	26.3	21.6	13.2	23.0	12.0	18.1	15.6	26.1
Bordeaux‡‡	98.8	98.2	100	89.2	97.0	-	-	-	-	-

† Failla *et al*., 1993a ; ‡ Bavaresco *et al*., 2010 ; § Failla *et al*., 1993b ; ¶ Weir and Cresswell, 1993 ; †† García-Escudero *et al*., 2013 ; ‡‡ Loué, 1990 ; d.w., dry weight ; -, data not available.

Table 2 - Dataset percentages of nutrient concentration in leaf blade at veraison of 'red Grenache' within the optimal values for different cultivars and/or regions.

Nutrient	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	Blade at veraison									
International references	$g \cdot 100g^{-1}$ d.w.					$mg \cdot kg^{-1}$ d.w.				
Trentino†	1.75-2.25	0.15-0.25	1.00-1.50	2.40-3.20	0.20-0.40	> 50	> 30	> 15	> 3	15-30
Italy‡	1.41-2.20	0.11-0.17	0.62-1.24	1.77-2.99	0.20-0.43	80-300	55-400	14-160	20-30	15-60
North-central Italy§	1.75-2.40	0.13-0.21	0.65-1.40	2.50-3.80	0.23-0.46	100-225	80-250	19-90	> 300	20-45
Australia¶	2.20-4.00	0.15-0.30	0.80-1.60	1.80-3.20	-	-	25-200	30-60	10-300	30-100
cv. Tempranillo (Rioja)††	2.19-2.29	0.15-0.16	0.77-0.91	3.10-3.34	0.38-0.46	134-164	99-124	16-19	117-221	34-40
Bordeaux‡‡	1.48-2.54	0.07-0.32	0.40-2.36	0.86-3.95	0.10-0.79	-	-	-	-	-
	Dataset percentages									
Number of cases	189	188	189	189	189	181	183	172	173	172
Trentino†	70.9	70.7	46.0	69.3	42.3	100	100	46.5	100	11.6
Italy‡	63.0	64.4	49.7	52.4	51.3	92.3	93.4	58.1	1.2	87.8
North-central Italy§	94.2	88.8	65.6	86.8	49.2	71.3	82.0	11.6	16.8	56.4
Australia¶	37.0	71.8	68.8	74.6	-	-	87.4	-	67.1	87.8
cv. Tempranillo (Rioja)††	18.5	27.1	7.4	22.8	18.5	13.3	23.0	23.8	14.5	23.3
Bordeaux‡‡	98.4	100	93.1	98.4	100	-	-	-	-	-

† Failla *et al*., 1993a ; ‡ Bavaresco *et al*., 2010 ; § Failla *et al*., 1993b ; ¶ Weir and Cresswell, 1993 ; †† García-Escudero *et al*., 2013 ; ‡‡ Loué, 1990 ; d.w., dry weight ; -, data not available.

For petiole at veraison, N ranged from 2.6 to 99.5 %, P from 17.5 to 96.3 %, K from 12.2 to 96.8 %, Ca from 3.7 to 100 %, Mg from 13.8 to 100 %, Fe from 10.2 to 99.2 %, Mn from 13.3 to 65.6 %, Zn from 4.7 to 51.6 %, Cu from 8.7 to 71.4 %, and B from 17.2 to 100 %. The largest difference between dataset optimal percentages was observed for N using 'Tempranillo' references (García-Escudero *et al.*, 2013) and Bordeaux references (Loué, 1990). Considering the Bordeaux references (Loué, 1990), 99.5 % of the 'red Grenache' dataset would be in an optimal nutritional status for N, while using the 'Tempranillo' references 97.4 % would be in a non optimal nutritional status, in spite of the fact that 'Tempranillo' references were suggested for the same region as the 'red Grenache' dataset.

2. Calculation of SR references

Nitrogen in both tissues and at both phenological stages, K, Ca and Mn in leaf blade at flowering and veraison, B in leaf blade at flowering, P, Ca and B in petiole at flowering, and Zn and B in petiole at veraison showed a normal distribution.

The Log-transformation to normal distribution was effective for P, Fe and Cu in leaf blade at flowering,

Table 3 - Dataset percentages of nutrient concentration in petiole at flowering of 'red Grenache' within the optimal values for different cultivars and/or regions.

Nutrient	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	Petiole at flowering									
International references	g·100g^{-1} d.w.					mg·kg^{-1} d.w.				
cv. Cabernet-Sauvignon (Brazil)†	0.96-2.93	0.13-0.88	1.04-3.00	1.02-2.84	0.22-0.66	20-130	104-524	37-141	5-518	20-81
cv. Italian Riesling (Brazil)†	0.70-1.93	0.11-0.65	1.12-3.17	1.00-2.70	0.19-0.83	24-345	85-501	35-153	5-638	20-74
cv. Tricandeira (Portugal)‡	0.68-1.38	0.34-0.64	1.08-2.62	1.21-1.91	0.54-0.98	13-25	38-434	14-38	6-10	27-35
Australia§	0.80-1.10	0.25-0.50	1.80-3.00	1.20-2.50	> 0.40	> 30	30-60	> 26	6-11	35-70
cv. Tempranillo (Rioja)¶	0.94-1.10	0.30-0.34	1.32-1.75	1.42-1.55	0.57-0.66	22-25	23-29	14-17	8.3-10	40-42
Bordeaux††	0.71-1.49	> 0.15	0.58-5.09	1.15-3.60	0.13-1.19	-	-	-	-	-
	Dataset percentages									
Number of cases	167	167	167	167	167	113	116	113	106	116
cv. Cabernet-Sauvignon (Brazil)†	97.0	100	85.6	97.6	24.6	95.6	17.2	0.0	98.1	100
cv. Italian Riesling (Brazil)†	82.0	95.8	80.8	93.4	41.9	86.7	21.6	1.8	98.1	100
cv. Tricandeira (Portugal)‡	35.9	76.0	80.2	32.9	50.3	13.3	70.7	59.3	61.3	1.7
Australia§	7.2	71.3	26.3	86.2	100	68.1	43.1	4.4	79.2	96.6
cv. Tempranillo (Rioja)¶	6.6	10.2	37.1	9.0	15.0	4.4	5.2	25.7	20.8	9.5
Bordeaux††	47.9	0.0	100	99.4	75.4	-	-	-	-	-

† Fráguas *et al.*, 2003 ; ‡ Pacheco *et al.*, 2010 ; § Robinson *et al.*, 1997 ; ¶ García-Escudero *et al.*, 2013 ; †† Loué, 1990 ; d.w., dry weight ; -, data not available.

Table 4 - Dataset percentages of nutrient concentration in petiole at veraison of 'red Grenache' within the optimal values for different cultivars and/or regions.

Nutrient	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	B
	Petiole at veraison									
International references	g·100g^{-1} d.w.					mg·kg^{-1} d.w.				
cv. Cabernet-Sauvignon (Brazil)†	0.61-1.09	0.07-0.64	1.07-4.74	1.26-3.67	0.21-2.08	14-91	77-2,063	26-97	14-667	21-70
cv. Italian Riesling (Brazil)†	0.57-1.14	0.06-0.50	1.51-3.91	1.10-4.92	0.31-1.13	18-98	87-1,035	37-107	28-2,233	22-70
cv. Tempranillo (Rioja)‡	0.47-0.51	0.10-0.13	1.14-1.68	1.86-2.09	0.78-0.95	23-27	44-74	19-24	16-26	35-38
Italy§	0.60-0.90	0.15-0.60	2.50-3.50	1.20-1.80	0.50-1.00	25-60	20-150	15-25	3-6	25-70
Bordeaux¶	0.31-1.00	0.07-0.71	0.36-6.91	0.84-5.05	0.22-2.59	-	-	-	-	-
	Dataset percentages									
Number of cases	189	189	189	189	189	128	128	128	126	128
cv. Cabernet-Sauvignon (Brazil)†	70.4	96.3	67.2	97.9	100	98.4	65.6	28.1	71.4	100
cv. Italian Riesling (Brazil)†	81.5	94.7	47.1	100	46	99.2	61.7	4.7	52.4	100
cv. Tempranillo (Rioja)‡	2.6	17.5	12.2	7.9	13.8	10.2	13.3	36.7	15.1	17,2
Italy§	68.3	65.1	17.5	3.7	37.6	72.7	50.0	51.6	8.7	98,4
Bordeaux¶	99.5	96.3	96.8	100	100	-	-	-	-	-

† Fráguas *et al.*, 2003 ; ‡ García-Escudero *et al.*, 2013 ; § Bavaresco *et al.*, 2010 ; ¶ Loué, 1990 ; d.w., dry weight ; -, data not available.

Table 5 - Sufficiency Ranges for leaf blade at flowering for 'red Grenache' grapevines.

Nutrient	Low	Below optimal	Optimal	Above optimal	High	S.P.‡
N (g·100 g^{-1} d.w.)	<2.83	2.83-2.97	2.97-3.10	3.10-3.25	>3.25	N.D.
P (g·100 g^{-1} d.w.)	<0.22	0.22-0.25	0.25-0.28	0.28-0.32	>0.32	Log
K (g·100 g^{-1} d.w.)	<0.84	0.84-0.94	0.94-1.02	1.02-1.11	>1.11	N.D.
Ca (g·100 g^{-1} d.w.)	<1.77	1.77-2.01	2.01-2.22	2.22-2.47	>2.47	N.D.
Mg (g·100 g^{-1} d.w.)	<0.23	0.23-0.28	0.28-0.33	0.33-0.38	>0.38	Perc.
Fe (mg·kg^{-1} d.w.)	<97	97-125	125-154	154-197	>197	Log
Mn (mg·kg^{-1} d.w.)	<80	80-103	103-123	123-145	>145	N.D.
Zn (mg·kg^{-1} d.w.)	<13	13-15	15-17	17-20	>20	Perc.
Cu† (mg·kg^{-1} d.w.)	<6	6-7	7-9	9-11	>11	Log
B (mg·kg^{-1} d.w.)	<53	53-62	62-71	71-80	>80	N.D.

† Concentration with a physiological meaning cannot be determined due to fungicide residues.
‡ Statistical procedure used to calculate SR subgroups: N.D., Normal distribution; Log, Transformation to Log_{10}; Perc., Percentiles.

Fe and B in leaf blade at veraison, P and Fe in petiole at veraison, and K, Fe, Mn, Zn and Cu in petiole at flowering.

Finally, P20, P40, P60 and P80 were calculated in cases where the Log-transformation to normal distribution was unlikely. That was the case for Mg and Zn in leaf blade at flowering and P, Mg, Zn and Cu in leaf blade at veraison. For leaf petiole, percentiles were calculated for Mg at flowering as well as for K, Ca, Mg, Cu and Mn at veraison. The calculated percentiles were used to define the 20 % and 60 % of the central data.

The sufficiency ranges for 'red Grenache' grapevine calculated for ten essential elements in leaf blade and petiole at flowering and veraison are shown in Tables 5 to 8.

3. Calculation of DOP references

The procedure to obtain the reference ranges was the same as the SR references. For DOP references, the reference value for each nutrient was the population mean or the mean value from the Log-transformation of the data to normal distribution. When the Log-transformation to normal distribution was ineffective, the DOP reference value was calculated using the median of the population, or percentile P50.

Table 9 shows the DOP reference values for 'red Grenache' macro and micronutrients in leaf blade and petiole at both flowering and veraison. The coefficient of variation (CV(%)) for each reference DOP index is also shown.

The CV(%) was higher for petiole than for blade for N, P, K, Mn and Zn at both phenological stages, Mg at flowering, and Ca and Fe at veraison. However, Ca showed similar CV(%) at flowering for both tissues. On the other hand, Cu and B at both phenological stages, Fe at flowering, and Mg at veraison showed higher CV(%) for blade than for petiole.

4. Comparison of 'red Grenache' references with respect to international references

A detailed comparison between the optimal ranges (central 20 % population) obtained for 'red Grenache' in Rioja with respect to the different international references found the following:

Leaf blade at flowering

'Red Grenache' references for leaf blade at flowering (Tables 1 and 5) showed higher values in the following cases: N and P when compared to Trentino (Failla *et al*., 1993a) and general Italian references (Bavaresco *et al*., 2010), and B when compared to references for the Trentino region and north-central Italy (Failla *et al*., 1993a, 1993b).

On the other hand, 'red Grenache' references showed lower values for P and Mg when compared to references for Australia (Weir and Cresswell, 1993), K when compared to Australia (Weir and Cresswell, 1993) and the Trentino region (Failla *et al*., 1993a), Ca when compared to vines from north-central Italy (Failla *et al*., 1993b), Zn when compared to Trentino, north-central Italy, Australian and Italian references (Failla *et al*., 1993a, 1993b; Weir and Cresswell, 1993; Bavaresco *et al*., 2010), and Cu when compared to north-central Italian, Australian and general Italian references (Failla *et al*., 1993b; Weir and Cresswell, 1993; Bavaresco *et al*., 2010).

With respect to the sensitivity of the references, Italian optimal ranges for all the nutrients studied had wider ranges than those obtained for 'red Grenache' in Rioja (Bavaresco *et al*., 2010).

Table 6 - Sufficiency Ranges for petiole at flowering for 'red Grenache' grapevines.

Nutrient	Low	optimal	Optimal	optimal	High	S.P.‡
N (g·100 g^{-1} d.w.)	<1.24	1.24-1.46	1.46-1.64	1.64-1.86	>1.86	N.D.
P (g·100 g^{-1} d.w.)	<0.34	0.34-0.41	0.41-0.46	0.46-0.52	>0.52	N.D.
K (g·100 g^{-1} d.w.)	<1.15	1.15-1.38	1.38-1.61	1.61-1.93	>1.93	Log
Ca (g·100 g^{-1} d.w.)	<1.75	1.75-1.98	1.98-2.19	2.19-2.43	>2.43	N.D.
Mg (g·100 g^{-1} d.w.)	<0.62	0.62-0.82	0.82-1.06	1.06-1.24	>1.24	Perc.
Fe (mg·kg^{-1} d.w.)	<26	26-32	32-38	38-46	>46	Log
Mn (mg·kg^{-1} d.w.)	<30	30-44	44-61	61-88	>88	Log
Zn (mg·kg^{-1} d.w.)	<11	11-13	13-16	16-20	>20	Log
Cu† (mg·kg^{-1} d.w.)	<7	7-8	8-9	9-11	>11	Log
B (mg·kg^{-1} d.w.)	<39	39-43	43-45	45-49	>49	N.D.

† Concentration with a physiological meaning cannot be determined due to fungicide residues.
‡ Statistical procedure used to calculate SR subgroups: N.D., Normal distribution; Log, Transformation to Log_{10}; Perc., Percentiles.

Table 7 - Sufficiency Ranges for leaf blade at veraison for 'red Grenache' grapevines.

Nutrient	Low	Below optimal	Optimal	Above optimal	High	S.P.‡
N (g·100 g^{-1} d.w.)	<1.99	1.99-2.09	2.09-2.18	2.18-2.28	>2.28	N.D.
P (g·100 g^{-1} d.w.)	<0.14	0.14-0.15	0.15-0.17	0.17-0.19	>0.19	Perc.
K (g·100 g^{-1} d.w.)	<0.92	0.92-1.14	1.14-1.34	1.34-1.56	>1.56	N.D.
Ca (g·100 g^{-1} d.w.)	<2.63	2.63-2.86	2.86-3.06	3.06-3.28	>3.28	N.D.
Mg (g·100 g^{-1} d.w.)	<0.21	0.21-0.29	0.29-0.40	0.40-0.47	>0.47	Perc.
Fe (mg·kg^{-1} d.w.)	<129	129-159	159-191	191-235	>235	Log
Mn (mg·kg^{-1} d.w.)	<91	91-124	124-153	153-187	>187	N.D.
Zn (mg·kg^{-1} d.w.)	<12	12-14	14-16	16-18	>18	Perc.
Cu† (mg·kg^{-1} d.w.)	<14	14-68	68-127	127-275	>275	Perc.
B (mg·kg^{-1} d.w.)	<32	32-39	39-45	45-54	>54	Log

† Concentration with a physiological meaning cannot be determined due to fungicide residues. ‡ Statistical procedure used to calculate SR subgroups: N.D., Normal distribution; Log, Transformation to Log_{10}; Perc., Percentiles.

In addition, the optimal ranges for N, P, K, Ca, Mg, Fe and Mn for north-central Italy (Failla *et al*., 1993b), Ca, Mg, Fe, Mn and Cu for the Trentino region (Failla *et al*., 1993a), the macronutrients for the Bordeaux region (Loué, 1990), as well as for N, P, Ca, Mg, Mn and B for Australia (Weir and Cresswell, 1993) also showed wider values than 'red Grenache' optimal ranges. For many of the other nutrients, besides showing wider ranges, their references were shifted compared to those of 'red Grenache' (Tables 1 and 5).

Finally, the comparison of 'red Grenache' references for blade with 'Tempranillo' references in the same winemaking region (García-Escudero *et al*., 2013) showed higher values for K, Fe, Mn and B and lower values for N, P, Ca, Mg, Zn and Cu (Tables 1 and 5).

Petiole at flowering

With respect to leaf petiole at flowering (Tables 3 and 6), 'red Grenache' references showed higher N values than Bordeaux, Australian and Portuguese ('Tricandeira') references (Loué, 1990; Robinson *et al*., 1997; Pacheco *et al*., 2010), higher Mg values than Portuguese ('Tricandeira') and Brazilian ('Cabernet sauvignon' and 'Italian Riesling') references (Pacheco *et al*., 2010; Fráguas *et al*., 2003), and higher Ca, Fe and B values than Portuguese references (Pacheco *et al*., 2010).

Furthermore, 'red Grenache' references showed lower K values than Australian references (Robinson *et al*., 1997), lower Zn values than Australian, Brazilian and Portuguese references (Robinson *et al*., 1997; Fráguas *et al*., 2003; Pacheco *et al*., 2010), and lower Mn values than Brazilian references (Fráguas *et al*., 2003).

On the other hand, international references showed wider optimal ranges than those for 'red Grenache'. In this sense, P, K, Mn and Cu for Portuguese references (Pacheco *et al*., 2010), N, P, K, Ca, Mg, Fe, Cu and B for Brazilian 'Cabernet sauvignon' and 'Italian Riesling' references (Fráguas *et al*., 2003), and all the studied macronutrients for Bordeaux

(Loué, 1990) showed wider optimal ranges than 'red Grenache' references (Table 6). Furthermore, for some nutrients, like Mg for Brazilian 'Cabernet - Sauvignon' and 'Italian Riesling' as well as N for Bordeaux, besides showing wider ranges, their references were shifted compared to those of 'red Grenache' (Tables 3 and 6).

Finally, 'red Grenache' references presented higher N, P, Ca, Mg, Fe, Mn and B values compared to 'Tempranillo' references for the same winemaking region in Spain (García-Escudero *et al*., 2013). On the other hand, Zn and Cu showed similar optimal ranges, while K showed a wider optimal range than 'red Grenache' references (Table 6).

Leaf blade at veraison

'Red Grenache' references for leaf blade at veraison (Tables 2 and 7) showed, for the optimal range, higher K, Ca and Cu values than Italian references (Bavaresco *et al*., 2010) and higher B values than Trentino references (Failla *et al*., 1993a). On the other hand, the same references showed lower N values than Australian references (Weir and Cresswell, 1993), lower P values than Trentino and Australian references (Failla *et al*., 1993a ; Weir and Cresswell, 1993), lower Zn values than Australian, Italian, and north-central Italian references (Weir and Cresswell, 1993 ; Failla *et al*., 1993b ; Bavaresco *et al*., 2010), and, finally, lower Cu values than north-central Italian references (Failla *et al*., 1993b).

Furthermore, 'red Grenache' had narrower optimal ranges than Trentino (Failla *et al*., 1993a), Italian (Bavaresco *et al*., 2010), Bordeaux (Loué, 1990), Australian (Weir and Cresswell, 1993) and north-central Italian references (Failla *et al*., 1993b) for all nutrients except Cu from Italy. Also for leaf blade at veraison, some of the nutrients showed shifted ranges from those obtained for 'red Grenache'. This was the case for B from Trentino (Failla *et al*., 1993a), K, Ca and Cu from Italy (Bavaresco *et al*., 2010), Zn and Cu from north-central Italy (Failla *et al*., 1993b), and N and Zn from Australia (Weir and Cresswell, 1993).

On the other hand, 'Tempranillo' references for the same region (García-Escudero *et al*., 2013) showed higher values for N, Ca, Mg, Zn and Cu, lower values for K, Fe, Mn and B, and similar P values as 'red Grenache' references (Tables 2 and 7). Furthermore, 'red Grenache' showed shifted ranges for all nutrients, except for P, from those obtained for 'Tempranillo'.

Leaf petiole at veraison

The Brazilian 'Cabernet Sauvignon' references (Fráguas *et al*., 2003) showed higher Zn values than 'red Grenache' while the 'Italian Riesling' references (Fráguas *et al*., 2003) showed higher Zn and Cu and lower Mg values than 'red Grenache' references (Tables 4 and 8). Finally, Italian references showed higher K values and lower Ca, Mg and Cu values than 'red Grenache' references (Bavaresco *et al*., 2010).

On the other hand, 'red Grenache' references showed narrower N, P, K, Ca, and Mg optimal ranges than Bordeaux, Italy, and Brazilian references for cvs. 'Cabernet-Sauvignon' and 'Italian Riesling' (Loué, 1990 ; Fráguas *et al*., 2003 ; Bavaresco *et al*., 2010). Furthermore, narrower Fe, Mn, Zn, Cu and B optimal ranges than those from Brazilian and Italian references were obtained, with the exception of Cu from Italy. Furthermore, for Zn from Brazilian 'Cabernet sauvignon' and Mg, Zn and Cu from Brazilian 'Italian Riesling', besides showing wider ranges, their references were shifted from those of 'red Grenache' (Tables 4 and 8).

Table 8 - Sufficiency Ranges for petiole at veraison for 'red Grenache' grapevines.

Nutrient	Low	Below optimal	Optimal	Above optimal	High	S.P.‡
N (g·100 g^{-1} d.w.)	<0.57	0.57-0.64	0.64-0.70	0.70-0.77	>0.77	N.D.
P (g·100 g^{-1} d.w.)	<0.12	0.12-0.17	0.17-0.22	0.22-0.30	>0.30	Log
K (g·100 g^{-1} d.w.)	<0.83	0.83-1.73	1.73-2.64	2.64-3.87	>3.87	Perc.
Ca (g·100 g^{-1} d.w.)	<2.19	2.19-2.40	2.40-2.52	2.52-2.77	>2.77	Perc.
Mg (g·100 g^{-1} d.w.)	<0.77	0.77-1.03	1.03-1.43	1.43-1.70	>1.70	Perc.
Fe (mg·kg^{-1} d.w.)	<30	30-37	37-45	45-55	>55	Log
Mn (mg·kg^{-1} d.w.)	<47	47-90	90-148	148-218	>218	Perc.
Zn (mg·kg^{-1} d.w.)	<16	16-21	21-25	25-29	>29	N.D.
Cu† (mg·kg^{-1} d.w.)	<10	10-18	18-39	39-66	>66	Perc.
B (mg·kg^{-1} d.w.)	<34	34-37	37-40	40-43	>43	N.D.

† Concentration with a physiological meaning cannot be determined due to fungicide residues.
‡ Statistical procedure used to calculate SR subgroups : N.D., Normal distribution ; Log, Transformation to Log_{10} ; Perc., Percentiles.

Table 9 - Nutrient concentration references for the Deviation from Optimum Percentage methodology in leaf blade and petiole at flowering and veraison, for 'red Grenache' grapevines.

	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu†	B
	Flowering									
	$g \cdot 100\ g^{-1}$ d.w.					$mg \cdot kg^{-1}$ d.w.				
Blade	3.04	0.268	0.976	2.12	0.309	139	113	16.3	8.14	66.5
CV(%)‡	8.28	22.8	16.2	19.7	26.5	45.1	34.2	28.4	36.9	24.7
Petiole	1.55	0.433	1.49	2.09	0.905	35.0	51.7	14.8	8.73	44.0
CV(%)‡	23.8	25.2	32.4	19.3	34.9	35.9	57.9	36.6	27.4	12.8
	Veraison									
	$g \cdot 100\ g^{-1}$ d.w.					$mg \cdot kg^{-1}$ d.w.				
Blade	2.14	0.162	1.24	2.96	0.347	174	139	14.9	89.5	41.9
CV(%)‡	8.08	17.4	30.8	13.1	39.1	36.2	41.1	25.9	193	29.7
Petiole	0.669	0.191	2.09	2.46	1.28	40.7	116	22.7	29.0	38.4
CV(%)‡	17.7	52.3	73.0	17.7	34.3	38.6	71.1	32.8	119	13.4

† Concentration with a physiological meaning cannot be determined due to fungicide residues.
‡ Coefficient of variation (%) or Median coefficient of variation (%).

Finally, 'red Grenache' references for leaf petiole at veraison (Tables 4 and 8) showed higher and shifted values for N, P, K, Ca, Mg, Fe, Mn and Cu and slightly higher and shifted values for Zn and B than 'Tempranillo' references (García-Escudero *et al.*, 2013).

DISCUSSION

1. Comparison of the 'red Grenache' dataset with respect to international references

It should be considered that all the surveyed grapevines included in the dataset were of good productive, sanitary and vegetative status. Thus, low diagnosis coincidences are incongruent with respect to a dataset that corresponds to representative 'red Grenache' vineyards.

The comparison shows important differences. For example, the biggest difference in the interpretation of the nutrient status of the 'red Grenache' dataset, using blade at flowering, was observed for B with respect to references from Trentino (Failla *et al.*, 1993a) and Australia (Weir and Cresswell, 1993). According to these references, the 'red Grenache' dataset would be in an optimal nutritional status for B when using references given for Australia (Weir and Cresswell, 1993), while its status would be non optimal when compared to Trentino references (Failla *et al.*, 1993a) (Table 1).

The high variability found for the dataset comparisons indicates the importance of using adequate references to determine the nutritional status of a determined cultivar. Thus, references must be adapted to each variety, soil and climatic condition, even within the same winemaking region. This fact has also been highlighted by other authors (Failla *et al.*, 1993a, 1995; Ciesielska *et al.*, 2002).

In general, the Bordeaux references (Loué, 1990) covered a greater number of optimal cases for all nutrients, while the Spanish references for 'Tempranillo' (García-Escudero *et al.*, 2013) covered fewer cases (Tables 1-4). This is due to a broader optimal range of nutrient concentration in Bordeaux references, while 'Tempranillo' references have more limited and perhaps sensitive optimal ranges, specific to a variety with an earlier maturation and senescence than 'red Grenache'.

2. SR and DOP reference concentrations

The database was split to assure that SR and DOP references would be specific to the combination *Vitis vinifera* L. cv. red Grenache grafted on Richter 110 and to reflect the total production and agro-climatic conditions of the region where references had been obtained.

The SR method divided the data population into five classes (*low*, *below optimal*, *optimal*, *above optimal* and *high*) considering the central range as the optimal nutritional status, whereas the higher and lower ranges of nutrient content with respect to the optimal range suggest that a corrective fertilization program should be considered.

However, the use of Cu-based products in vineyards for phytosanitary purposes prompts the adsorption processes of Cu by the leaf surface. This adsorption increased the total Cu concentration analyzed and therefore a Cu reference with a real physiological meaning for DOP as well as for SR was not obtained. This fact is critical at veraison as by then the phytosanitary applications have already started. The CV(%) for Cu's DOP references shows the high variability found for both tissues at veraison (Table 9).

Each nutrient reference for the DOP method is the mean or centred value, which represents the dataset population for each nutrient as a whole (Table 9). Concentrations below or above the DOP reference value, for each nutrient, produce negative or positive DOP indexes, respectively, and therefore a corrective fertilization program or a reduction of the nutrient in the fertilization programs must be considered.

The lower CV(%) of DOP references for blade suggests that blade has a higher sensitivity than petiole to show deficiencies or excesses of N, P, K, Mn, and Zn at both flowering and veraison stages, Mg at flowering, and Ca and Fe at veraison. On the other hand, petiole has higher sensitivity than blade to show Cu and B deficiencies or excesses at both phenological stages, as well as to show deficiencies of Fe at flowering and Mg at veraison.

Therefore, a technician can analyze leaf blade or petiole, or analyze both tissues and evaluate each nutrient in the most appropriate tissue for a more accurate nutritional diagnosis of 'red Grenache' according to the corresponding reference, DOP or SR in this case, for the phenological stage of the sampling.

In addition, SR or DOP references will be less reliable when the regional conditions of the studied vineyard are different from the ones where references were originally obtained (Failla *et al.*, 1995 ; Robinson, 2005 ; García-Escudero *et al.*, 2013) or if references are employed for the nutritional diagnosis of other varieties. The proposed references will be less accurate when the vineyard conditions differ from the ones selected to create the dataset.

3. Comparison of 'red Grenache' references with respect to international references

The sensitivity of the references could be estimated by means of the width of the optimal ranges. In summary, the comparison (detailed in the Results section) of international references (Tables 1-4) with respect to 'red Grenache' SR references from Rioja (Tables 5-8) showed, in general, wider optimal ranges for international references for both tissues and phenological stages. Furthermore, important differences were observed between 'red Grenache' DOP references (Table 9) with respect to the nutrient concentration values proposed by those international references (Tables 1-4). Both considerations mean that the use of non-appropriate references has a lower sensitivity to detect deficiencies or excesses in 'red Grenache'. Furthermore, Spanish references for 'Tempranillo' from the same winemaking region also showed important differences with respect to 'red Grenache' references, reinforcing the need to establish specific references for the different winemaking regions and even between different cultivars within a geographic area.

CONCLUSION

SR and DOP references are proposed for the combination *Vitis vinifera* L. cv. red Grenache grafted on Richter 110 in order to reflect the nutritional status of vineyards under similar production and agro-climatic conditions as the region where references have been obtained. The proposed references will be a useful tool to assess the nutritional status of 'red Grenache' around the world, but they will be less accurate when the rootstock, the variety or the regional conditions of the studied vineyard differ from the ones selected to create the dataset ; therefore, a proper use of the references requires a technician to evaluate the differences regarding soil, climate, rootstock, as well as other factors.

Finally, references for 'red Grenache' will be enhanced in the future because of a continuous increase of the foliar database as well as the introduction of new criteria in the calculation, such as must quality and/or different yield ranges. In this sense, a bigger database would make it possible to obtain more specific references, such as references for rainfed and irrigated vineyards or references that take into account different training systems, soil managements and the age of the vines.

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