Received: 23 August 2020 | Revised: 7 March 2021 | Accepted: 10 March 2021

DOI: 10.1002/pld3.319

ORIGINAL RESEARCH

Water stress assessment on grapevines by using classification and regression trees

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Funding information
This research was funded by the University Rovira i Virgili, Biochemistry Department, Viticulture Research Group. Partial support for this work has been received from the Agency for Management of University and Research Grants (AGAUR) of the Government of Catalonia (grant 2017 SGR 705).

Abstract

Multiple factors, such as the vineyard environment and winemaking practices, are known to affect the development of vines as well as the final composition of grapes. Water stress promotes the synthesis of phenols and is associated with grape quality as long as it does not inhibit production. To identify the key parameters for managing water stress and grape quality, multivariate statistical analysis is essential. Classification and regression trees are methods for constructing prediction models from data, especially when data are complex and when constructing a single global model is difficult and models are challenging to interpret. The models were obtained by recursively partitioning the data space and fitting a simple prediction model within each partition. The partitioning can be represented graphically as a decision tree. This approach permitted the most decisive variables for predicting the most vulnerable vineyards and wine quality parameters associated with water stress. In Priorat AOC, Carignan grapevines had the highest water potential and abscisic acid concentration in the early growth plant stages and permitted vineyards to be classified by mesoclimate. This information is useful for identifying which measurements could most easily differentiate between early and late-ripening vineyards. LWP and T_s during an early physiological stage (pea size) permitted warm and cold areas to be differentiated.

KEYWORDS

ABA, anisohydric, carignan, classification and regression trees, isohydric, water stress

1 | INTRODUCTION

Water stress on vine plants induces the synthesis of secondary metabolism. Around veraison, water deficit stress causes a significant increase in the abscisic acid (ABA) level in fruit zone leaves (Okamoto et al., 2004) and berries (Coombe & Hale, 1973; Düring & Allenweldt, 1980). ABA plays an important role in the regulation of growth and the ripening of vines. Lack of water in the soil and elevated temperatures induce the synthesis of ABA in the roots, followed by its translocation to the leaves, where it rapidly alters the osmotic potential of stomatal guard cells, causing them to shrink and the stomata to close. Stomatal closure reduces transpiration and thus prevents further water loss from the leaves during periods of low water availability. Around veraison, ABA levels in grapes

One-sentence summary: Classification and regression trees is a very easy-to-use statistical tool for vineyard parameters characterization from high variability data.

increase significantly, along with the stimulation of ripening and phenolic synthesis, but decrease during the final stage of berry ripening (Bondada & Shutthanandan, 2012; Palejwala et al., 1985; Soar et al., 2006; Wheeler et al., 2009). Abscisic acid may be translocated from the sites of biosynthesis, such as roots and leaf vascular tissues, to the guard cells. Recent identification of multiple transmembrane ABA transporters indicates that the movement of this hormone within plants is actively regulated in an intercellular network (Kuromori et al., 2018).

Regulation of water deficits has often been used to balance grapevine vegetative and reproductive growth to control berry quality (Chaves et al., 2010). Analysis of the phenolic composition in wine is essential for establishing quality parameters related to water stress, as some studies have shown that ABA is involved in the mechanisms controlling the synthesis of anthocyanins and promotes the synthesis of tannins accumulating in skin (Lacampagne et al., 2010) ABA synthesis depends on different factors promoting water stress; plant water physiology is affected by various environmental factors (e.g., topography, soil water-holding capacity, temperature, rainfall, and vapor deficit pressure), plant vigor, and cultural practices, such as irrigation techniques and fertilization programs (Downey et al., 2004; Jackson & Lombard, 1993) and by scion/rootstock interaction with soil type (Lavoie-Lamoureux et al., 2017), Grenache is highly influenced by vigor, because anthocyanin accumulation is favored in balanced, high-vigor vines, whereas in Carignan, the anthocyanin content varies under the combined effects of vigor, rootstock, berry size, and vintage (Edo et al., 2014).

Appropriate statistical tools are required for identifying the factors that have the strongest effects on quality and stress during growth (plant) and maturation (grape). Predictors, such as linear or polynomial regressions, are global models, where a single predictive formula is applied over the entire dataset. However, when the data interact in complex, nonlinear ways, assembling a single global model is challenging. Classification-type problems can be resolved when a categorical dependent variable (e.g., class and group membership) is predicted from one or more continuous and/or categorical predictor variables. Generally, the purpose of analyses involving tree-building algorithms is to determine a set of *if–then* logical (split) conditions that permit accurate prediction or classification of the data.

The aim of this study was to evaluate the efficacy of a multivariate nonparametric technique of classification and regression trees (CART) for identifying and selecting the most important factors affecting water stress in vineyards with a heterogenic orography (e.g., leaf water potential [LWP], concentration of ABA, surface leaf temperature [T_s]); analyze the effect of these interactions on final grape and wine quality (e.g., composition of anthocyanins and procyanidins); and improve the rapidity with which ABA can be measured in grapevine leaves. The heterogeneity of the vineyards in the Priorat wine region requires the collection of a considerable amount of data and more robust statistical tools to better understand the factors affecting water stress in vineyards. Because of the increasing drought and higher temperatures occurring in the Priorat, the Priorat is highly vulnerable to future climate change. Here, we explore applications of multivariate nonparametric classification techniques such as CART, a type of decision tree technique (Breiman et al., 1984), given that traditional methods are not appropriate for analyses because of the characteristics of the variables studied.

TABLE 1 Values of predawn leaf water potential (PLWP, Ψ_{PLWP} [MPa]) and midday leaf water potential (MLWP, Ψ_{MLWP} [MPa]) for Sites 1, 2, 3, 4, and 5 at two different stages of growth—pea size (PS) and veraison (V)—at predawn and midday

	Pea size (PS)		Veraison (V)	
Site	Ψ_{PLWP} Predawn	Ψ_{MLWP} Midday	Ψ_{PLWP} Predawn	Ψ_{MLWP} Midday
1	−0.33 (0.04)	−1.29 (0.05)	−0.47 (0.12)	−1.38 (0.07)
2	−0.43 (0.08)	−1.21 (0.16)	−0.54 (0.13)	−1.44 (0.08)
3	−1.43 (0.01)	−1.48 (0.04)	−0.82 (0.21)	−1.76 (0.07)
4	−1.27 (0.04)	−1.39 (0.05)	−0.47 (0.05)	−1.58 (0.06)
5	−1.28 (0.03)	−1.50 (0.00)	−0.92 (0.08)	−1.50 (0.04)

Note: Values are mean and standard deviation.

TABLE 2 Values for abscisic acid concentration (ABA) for Sites 1, 2, 3, 4, and 5 at two different stages of growth—pea size (PS) and veraison (V)—at predawn and midday

	Pea size (PS)		Veraison (V)	
Site	[ABA] Predawn	[ABA] Midday	[ABA] Predawn	[ABA] Midday
1	152.8 (4.7)	195.0 (33.4)	162.8 (7.6)	243.5 (13.1)
2	181.1 (21.4)	226.4 (5.9)	92.5 (8.7)	115.5 (3.6)
3	152.0 (17.3)	229.0 (42.2)	97.3 (15.5)	89.9 (8.6)
4	211.8 (5.5)	423.0 (80.7)	83.7 (2.4)	134.8 (38.7)
5	196.3 (5.9)	400.1 (19.8)	114.9 (12.7)	178.8 (9.3)

Note: Mean and standard deviation.

2 | RESULTS

2.1 | LWP and ABA

LWP and ABA measurements are shown in Tables 1 and 2. After characterizing differences in variability through a nonparametric Kruskal–Wallis test (Table 3) at a significance level of 5%, Pearson correlations between the measured variables and their significance (Table 4) were calculated. The classification of sites was captured by the Classification and Regression Trees (CART) to help identifying key variables in the data. The most meaningful predictors were used to create the tree. Plant, grape and wine data were collected to evaluate the interactions. However, to obtain reliable classification and regression trees, a previous selection of child nodes was completed using the easiest-to-measure variables in the field and the easiest-to-analyze variables in the laboratory. Each round of data is known as 'nodes'. Each node will have an *if–else* clause based on a labeled variable. Based on that question each instance

TABLE 3 Analysis of the differences between groups using the nonparametric Kruskal–Wallis test

Conditions	Hour	Phenological stage	*p* value
Leaf water potential	Predawn	PS	.014
	Midday	PS	.014
	Midday	V	.017
Abscisic acid content	Predawn	PS	.019
	Midday	V	.017
Leaf surface temperature	Predawn	PS	.012
Total anthocyanins		Wine	.019
Glycosylated anthocyanins		Wine	.014
Acetyl glycosylated anthocyanins		Wine	.011
Berry weight			.009
Total leaf area/kg		V	.024
		RP	.019

Abbreviations: PS, pea size; RP, ripeness; V, veraison.

of input will be directed/routed to a specific leaf-node which will tell the final prediction. The tree depth is chosen as the most number of levels desired in the decision tree. The first node is split based on the most important predictor, then the following child nodes are broken down to separate out the next variable. Entering a value, the program sets the minimum number of cases an internal node is to be split. Three times terminal node limits allow a reasonable number of splitters.

2.1.1 | CART: WATER STRESS AND PLANT GROWTH

Plant growth parameters that differed significantly (*p* value ≤ .05) between plots were berry weight and total leaf area/kg (TLA/kg) at the veraison (V) and ripening (RP) stages. Water stress indicators that differed significantly between plots were LWP and [ABA] at pea size (PS) and veraison(V) and surface temperature (T_s) at pea size (PS). Pearson correlations revealed that LWP at PS measured at 8:00, ABA at V measured at 14:00, and T_s at PS measured at 8:00 were negatively correlated with the synthesis of anthocyanins in wine for all anthocyanin families (acylated and non-acylated). LWP and T_s showed stronger correlations when these parameters were measured earlier in the day (8:00) or at the beginning of the vegetative cycle (PS). The same variables—LWP at PS measured at 8:00, ABA at V measured at 14:00, and T_s at PS measured at 8:00—were positively correlated with TLA/kg V.

As a result from this the CART, LWP at PS measured at 8:00 was the most important predictor allowing to create the first node that separated early mesoclimates (Nodes 6 and 7) from late mesoclimates (Nodes 4 and 5). Nodes 2 and 3 were dependent on

TABLE 4 Pearson correlation matrix

Pearson correlation matrix	[ABA] (ng/g) predawn PS	[ABA] (ng/g) midday V	Ψ_{PLWP} (MPa) predawn PS	Ψ_{MLWP} (MPa) midday PS	Ψ_{MLWP} (MPa) midday V	Ts (°C) predawn PS	Berry weight (g)	TLA/kg V	TLA/kg RP	ANT (mg/L) wine	A-G (mg/L) wine	A-AG (mg/L) wine
[ABA] predawn PS	1											
[ABA] midday V	−0.143	1										
Ψ predawn PS	−0.283	0.489	1									
Ψ midday PS	−0.011	0.145	**0.798**	1								
Ψ midday V	0.116	**0.696**	**0.796**	**0.574**	1							
Ts predawn PS	−0.226	0.451	**0.935**	**0.763**	**0.702**	1						
Berry weight	−0.228	0.316	**0.783**	**0.697**	0.492	**0.917**	1					
TLA/kg V	**−0.516**	**0.627**	**0.738**	0.437	**0.578**	**0.703**	**0.566**	1				
TLA/kg RP	−0.304	**0.682**	0.015	−0.262	0.190	−0.006	−0.161	0.478	1			
ANT-wine	0.319	**−0.703**	**−0.588**	−0.408	−0.461	**−0.737**	**−0.752**	**−0.699**	−0.451	1		
A-G-wine	0.278	**−0.680**	**−0.576**	−0.380	−0.461	**−0.752**	**−0.776**	**−0.661**	−0.407	**0.986**	1	
A-AG-wine	**0.610**	**−0.628**	**−0.658**	−0.411	−0.393	**−0.732**	**−0.738**	**−0.846**	−0.449	**0.917**	**0.882**	1

Note: Bold values are different from 0 at a significance level (α) of 0.05.

Abbreviations: A-AG, acetyl glycosylated anthocyanins; ABA, abscisic acid; A-G, glycosylated anthocyanins; ANT, total anthocyanins; PS, pea size; RP, ripeness; TLA, total leaf area; T_s, leaf surface temperature; V, arount veraison; Ψ_{MLWP}, midday leaf water potential; Ψ_{PLWP}, predawn leaf water potential.

ABA at PS (late mesoclimate) and V (early mesoclimate). However, obtaining a partition of the five sites [ABA] at V was decisive and resulted in the generation of Nodes 8 and 9. As a consequence, the sites with the highest probability of being classified with LWP values ≤−0.863 (8:00 at PS) were the parcels located in the town of Molar (Sites 1 and 2). Hence, Site 1 had levels ≥ABA 175.9 ng/g (14:00 at V) (Figure 1). Site 3, within the late mesoclimate area, had a lower probability of having ABA ≤ 183.9 ng/g (morning at pea size) PS. Fewer factors differentiated Site 3 (gray) from the other sites; it was thus separated in an early node as in Sites 1 and 2 (blue and red) of the early mesoclimate area (Figure 1).

2.1.2 | CART: ABA, LWT, AND T_s

The most significant variables for characterizing and classifying the observations were [ABA], LWP, and T_s. T_s was selected given that it had a direct relationship (positive Pearson correlation) with the vegetative growth parameters of TLA/kg and berry size. The Pearson correlation produced a clear classification tree (Figure 2) based on the T_s, at the root node, it generated three child nodes (2, 3, and 4). This first classification by T_s at PS measured at 7:00 resulted in a purity of 100% for Site 4, but the T_s at PS measured at 12:00 was clearly the most important variable for Sites 5 and 6 under a second child node classification. However, the early sites (1 and 2) were differentiated by [ABA] at PS measured at 8:00.

Although many authors have described the effect of T_s on the quality of grapes during the ripeness period (Greer & Weedon, 2013; Spayd et al., 2002; Van Leeuwen et al., 2009), the analysis of the tree shows the magnitude of the effect of T_s from the early stage of PS. Measurements taken at 8:00 at PS were more likely to have values of T_s ≤ 22.0°C in the late mesoclimate area. Child Node 4 indicates that Sites 1 and 2 had a high probability of being classified within the temperature range 22°C ≤ T_s ≤ 24°C (8:00 at PS). Using the CART greatly facilitates the characterization of the importance of the classification of vineyards, especially in the late area (Sites 3, 4, and 5). Furthermore, Sites 3 and 5 were located in equivalent positions in the tree (purity 50%); thus, the differentiation of both plots from other plots depended on the same factors. Remarkably, both Site 3 and Site 5 had similar TLA and thus greater water loss. (Figure 2).

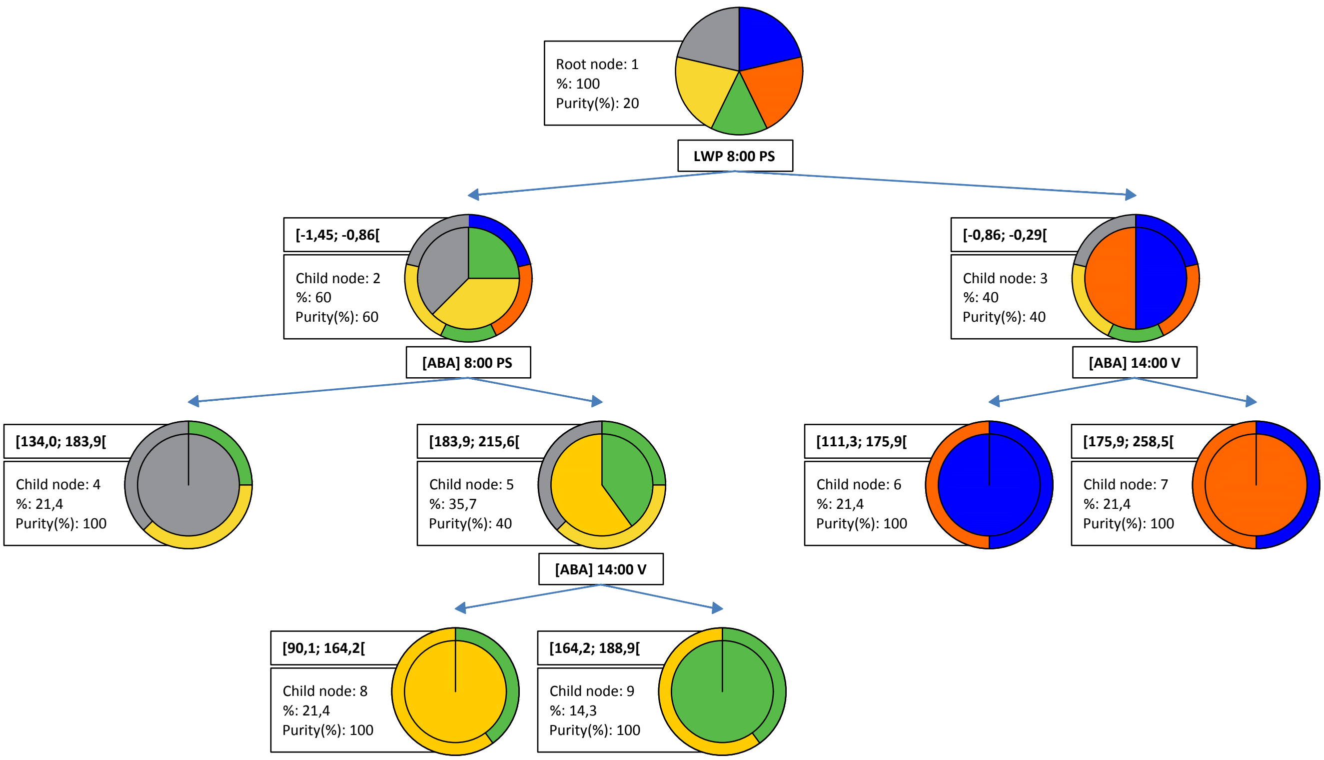

FIGURE 1 Classification and regression trees by water stress indicators (LWP, ABA, and T_s). Site 1 (red), Site 2 (blue), Site 3 (gray), Site 4 (orange), and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child nodes are broken down to separate out the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each color represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the *if–else* question. The circles on the right branch correspond to those vineyards with higher values and those on the left to those with lower values, in answer to the *if–else* question (values are shown in brackets)

2.1.3 | CART: ANTHOCYANINS IN WINE QUALITY

In this CART analysis, Pearson correlations of plant parameters and wine composition in each site were calculated. Both LWP at PS measured at 8:00 and LWP at V measured at 14:30 were correlated with ANT (mg/L), A-G (mg/L), and A-AG (mg/L). However, lower correlation coefficient values were obtained for LWP at V measured at 14:30 pm. Despite the difficulty of establishing direct links between plant parameters (TLA/kg at V) and wine composition (anthocyanins), robust correlations were found for T_s at PS measured at 7:00 and wine anthocyanins (non-acylated and acylated). The most significant relationship was for the correlation between TLA/kg V and A-AG (mg/L).

Based on the easy-to-measure parameters in the vineyard, such as T_s and the ratio of leaf area and production at V (TLA/kg V), we could characterize the relationship between the water status of plants and plant growth to the quality of the final wine product. This classification of plots allowed us to determine patterns of heterogeneity between plots. Thus, the CART classifies sites through the nodes to distinguish among different vineyards. (Figure 3).

The tree shows that LWP (Node 1) at PS permitted the differentiation of early (EM) and late (PO) sites. Values within the range −1.45 ≤ LWP ≤ −0.862 described the late ripeness sites (4, 5, and 6), while the range −0.863 ≤ LWP ≤ −0.290 classified the warmest sites (1 and 2). In the late mesoclimate area (Node 2), sites were separated by anthocyanins; Sites 3, 4, and 5 were classified together by Node 5 and were primarily influenced by the LWP at 14:30 in V. This finding suggests that the topography of the vineyard location, as well as the climate and soil type, had an important influence on wine quality. However, the parameter that classifies vineyards was ABA at 14:00 V (≤175.9 ng/g) by Node 3 and was necessary for divided Sites 1 and 2 (early mesoclimate). Thus, LWP did not affect the phenolic content of the wines.

3 | DISCUSSION

Measurements of the distribution of soil water revealed that the differences detected among the five sites reflected heterogeneity in soil particle size, depth, and texture. Sites 1 and 2 (El Molar) on a clayey soil had a higher water-holding capacity, than that of Sites 3, 4, and 5 (Porrera), which were steep with more stones and soil was primarily composed of larger elements. Thus, the vines in the town of El Molar (Sites 1 and 2, early mesoclimate) had more available water than those in Porrera (Sites 3, 4, and 5, late mesoclimate), despite the lower rainfall recorded during the cycle. Predawn leaf water potential (PLWP) reflects soil water availability as perceived by the plant and midday leaf water potential (MLWP) measures leaf water potential under maximum daily water demand. Therefore the higher soil water content at Site 1 and Site 2 led to more vigorous plants because LWPs were less negative. In Porrera, because of the lower water retention in soils, the plants had more negative LWPs than those in Molar. In addition, at approximately the pea size phenological stage, water transpiration by leaves was higher and LWPs showed more negative values because of the low soil water

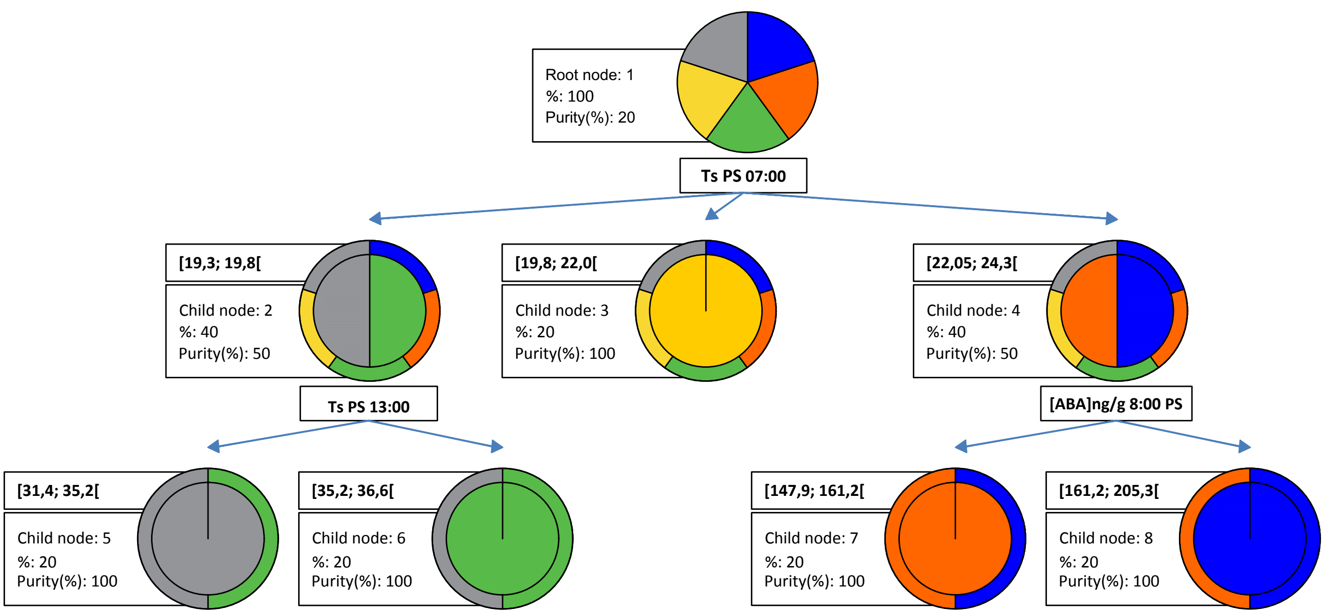

FIGURE 2 Classification and regression trees by T_s (surface canopy temperature). Site 1 (red), Site 2 (blue), Site 3 (gray), Site 4 (orange), and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child nodes are broken down to separate out the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each color represents the data from a single vineyard. The inner circle is the percentage that results from answering the *if–else* question. The circles on the right branch correspond to those vineyards with higher values and those on the left to those with lower values, in answer to the *if–else* question (values are shown in brackets)

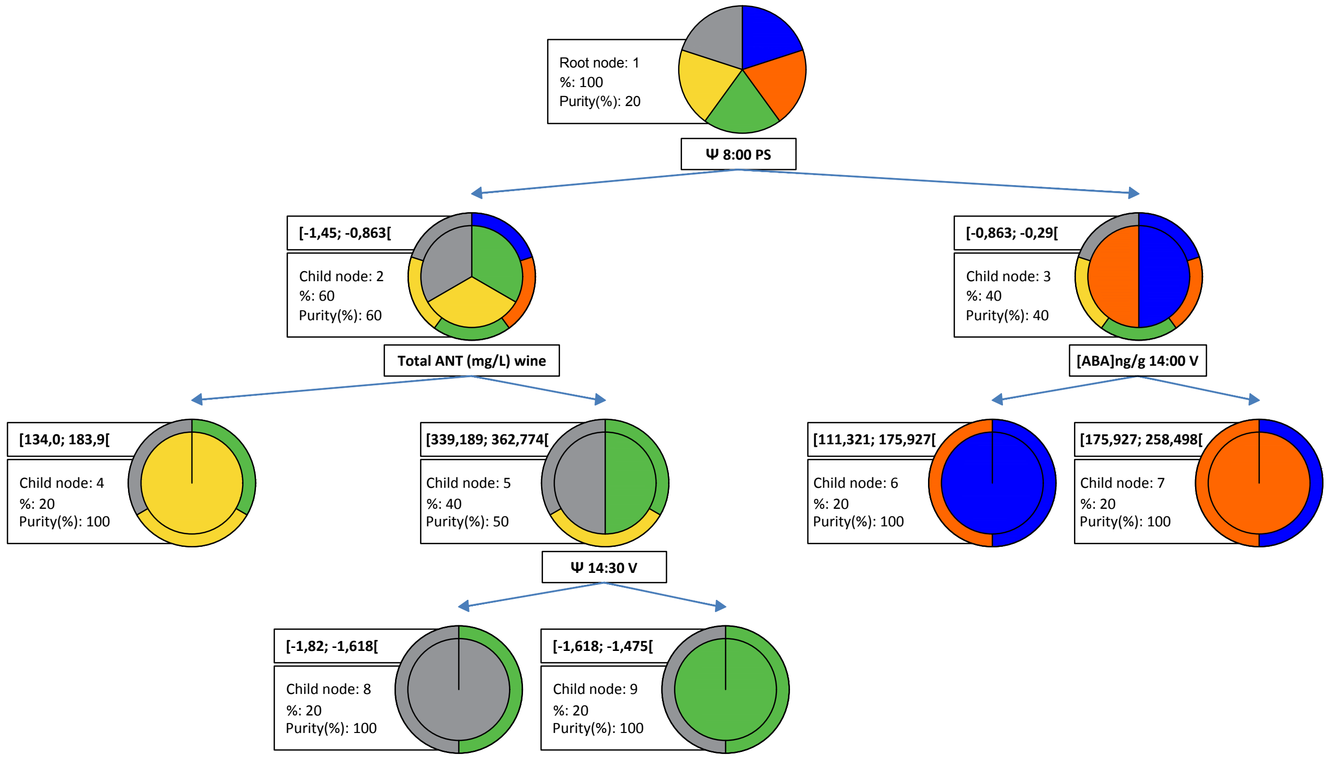

FIGURE 3 Classification and regression trees by total anthocyanins. Site 1 (red), Site 2 (blue), Site 3 (gray), Site 4 (orange), and Site 5 (green). Root node represents the entire population and splits based on the most important predictor, then the following child nodes are broken down to separate out the next parameters. The outer circle represents the data percentages of the previous step per each vineyard, where each color represents the data from a single vineyard. The inner circle pie is the percentage that results from answering the *if–else* question. The circles on the right branch correspond to those vineyards with higher values; those on the left to those with lower values, in answer to the *if–else* question (values are shown in brackets)

content in the stony and poor soil. It is known that *Vitis* genotypes show either an isohydric or anisohydric response to water stress. In isohydric cultivars, strong control of stomatal conductance by ABA reduces transpiration, obviates decreases in water potential, and delays the onset of stress tolerance mechanisms. In contrast, weak ABA control of stomatal closure does not avoid midday decreases in water potential in anisohydric grapevines (Lovisolo et al., 2010). In addition, during periods of low water availability and higher transpiration water demand, many authors have observed that a hydraulic signal can also have a controlling effect on stomatal conductance, and this also relates to both patterns, isohydric species maintain relatively stable LWPs precisely because of their more strict stomatal control, whereas anisohydric species would show a looser regulation of transpiration. What is more, the degree of isohydry can be related to a reduced soil water availability (lower, more negative soil water potential, Ψ_{soil}) may affect plant conductance in two ways, by lowering its hydraulic conductance (K_H) and/or its leaf conductance (g_{Leaf}). These reductions, have opposite effects on the water potential difference through the plant ($\Delta\Psi = |\Psi_{Leaf} - \Psi_{soil}|$), whereas lower K_H increases $\Delta\Psi$, lower g_{Leaf} decreases $\Delta\Psi$ (Martínez-Vilalta & García-Forner, 2017; Martínez-Vilalta et al., 2014). Thus, there is a tight coordination between hydraulic and water vapor transport at the plant level (Sperry & Love, 2015).

Parameters that best discriminated between sites were LWP and ABA content, followed by berry size and anthocyanin concentration. Around veraison, higher correlations between LWP and ABA content were obtained. After analysis of the Pearson correlations, the best results were obtained for the veraison phenological stage where vapor pressure deficit (VPD) is lower. ABA concentrations in Carignan vines at different sites (early (1 and 2) and late (3, 4, and 5)) are shown in Table 4. Higher concentrations of ABA were observed in all vineyards when measurements were taken at noon. This observation reflects increased water stress in all plots and confirmed measurements of LWP. It also established a direct correlation between the concentration of ABA and LWP (R^2 = .918). The strongest correlations were observed for the first measurements in the morning, while measurements at noon showed greater dispersion, R^2 (.7175). Thus, the CART analysis could distinguish among sites of the later mesoclimate region based on ABA at pea size stage.

In Figure 1, PLWP at pea size separated sites within mesoclimate and reached values of −0.86 for the early and −1.45 for the late mesoclimate. Around veraison, ABA concentration classified vineyards in the warmest area with values of 258 ng/g in Site 1 and 175 ng/g in Site 2. In the coldest area, the values were lower and did not separate at such wide intervals. At Site 3, ABA concentration did not exceed 183 ng/g at pea size; instead, values were higher in Sites 4 and 5 but did not differentiate vineyards. Values for these plots at veraison were lower than in pea size; Site 3 had values as high as 164 ng/g, and Site 5 had values as high as 188 ng/g. The three most similar sites in ABA at veraison at noon were Sites 2, 4, and 5. Thus, the ABA concentration at veraison is important for differentiating most of the plots, including Sites 1, 2, 4, and 5.

In Figure 2, T_s at pea size measured at predawn permitted separation by temperature ranges and isolated Site 2 with temperatures between 19.8°C and 22°C. The early sites were separated by ABA at pea size at predawn (with higher values in Site 2, considering that Site 1 had a rocky soil, while Site 2 was composed by finer elements). Plots of the coldest area were only separated by T_s at pea size at noon. Site 5 was located at higher elevation and experienced higher temperatures at noon (36.6°C) than Site 3 (35.2°C). T_s at veraison did not provide useful information because the plots experienced similar levels of stress. Thus, the characterization of the plots by T_s can be predicted at pea size but not around veraison.

In Figure 3, PLWP at pea size separated sites with different mesoclimates. Sites 1 and 2 differed in ABA around veraison at noon (Figure 1). The ABA concentration in Site 1 was twice that of Site 2; thus, these plots did not differ in the concentration of anthocyanins unlike the colder sites. Sites in the cold mesoclimate were classified by the anthocyanins in wine. Although there was a strong correlation with anthocyanins in grapes, wine correlated with other variables (as evidenced by Pearson correlations greater than 0.7). Plants at Site 2 were the least vigorous with anthocyanin values less than 339 m/L. Because plants at Sites 3 and 4 showed more vigor, the effect that distinguished the plots was MLWP at veraison, as the water stress was increased in Site 3 (LWP of −1.82) and Site 4 (LWP of −1.6).

Even if the action of ABA in occlusive cells is complex and not yet fully understood, *Vitis* genotypes apparently exhibit different levels of drought adaptation that differ in key steps involved in ABA metabolism and signaling (Rossdeutsch et al., 2016). In general, *Vitis vinifera* varieties, displayed more pronounced responses to water deficit in comparison to other *Vitis* genotypes. Moreover, Dal Santo et al., (2016) proposed a cause–effect link between the physiological grapevine plant conditions and the intensity of the gene expression changes. Finally, in regards to grape composition, many key genes (VvMybA1 and VvUFGT) of the flavonoid biosynthetic pathway are also up-regulated during ripening, resulting in a berry quality increase (Ferrandino & Lovisolo, 2014). ABA accumulation and the induction of flavonoid biosynthesis increase the quality of berries by facilitating the accumulation of secondary metabolites, especially polyphenols. Under water stress, polyphenolic concentrations increase in berries both in isohydric varieties, such as *Grenache* (Coipel et al., 2006), *Tempranillo* (Santesteban et al., 2011), *Manto negro* (Medrano et al., 2003), and in anhysohydric varieties, such as *Cabernet Sauvignon* (Bindon et al., 2008; Kennedy et al., 2002), *Cabernet Franc* (Matthews & Anderson, 1988), and *Muscat of Alexandria* (Dos Santos et al., 2007), with different temporal dynamics related to ABA induction. Aquaporins are another target for ABA to regulate both water and carbon fluxes. ABA affects aquaporin regulation in response to abiotic stresses (Kaldenhoff et al., 2008) by modulating their gene expression and protein abundance or activity, affecting in cellular water relations and cell metabolism in response to water stress. Aquaporins can be modulated at several levels, via transcription, translation, trafficking and gating (opening and closing of the pore) and by environmental and developmental factors (Chaumont & Tyerman, 2014), such as: irradiation (Lopez et al., 2013; Prado et al., 2013), transpiration (Laur & Hacke, 2013; Sakurai-Ishikawa et al., 2011), circadian rhythms (Hachez et al., 2008), abscisic acid (ABA) feeding (Pantin et al., 2013; Shatil-Cohen et al., 2011), auxin feeding (Péret et al., 2012) and shoot wounding (Sakurai-Ishikawa et al., 2011; Vandeleur et al., 2014). Coupled with that, Castellarin et al., (2007) showed that water stress favored the accumulation of more hydroxylated and methylated anthocyanins (peonidin 3-O-glucoside and malvidin 3-O-glucoside). In addition, the degradation of anthocyanin would probably be induced by high temperatures with an oxidative stress leading to the formation of H_2O_2, with the subsequent induction of peroxidases and of oxidoreduction enzymes (Mori et al., 2007). In contrast, little is known about the impact of temperature on proanthocyanidin accumulation in grape skins; berries are able to compensate the initial effects of temperature on proanthocyanidin biosynthesis resulting in similar concentration of proanthocyanidin at harvest (Cohen et al., 2012).

Overall, the effect of variables on the classification of the trees was closely tied to the water scarcity of the plants. In viticulture science it is of particular importance to evaluate whether the relationships between physiological parameters fitted to data through these powerful statistical methodologies. In addition, some authors (Brillante et al., 2017) have shown that well-trained machine-learning models can be used to capture the essential relationships between plant physiology and the environment. As an example, Brillante et al., (2016) have for the first time modeled grapevine water stress. This models will be important to design experiments and provide with validation tests to demonstrate the efficiency of the models.

4 | CONCLUSIONS

To assess water stress in grapevines, both LWP and concentration of ABA are important for characterizing the physiology of the growing season and its effects on phenol grape quality. A methodology that permits rapid and accurate responses to ABA to be determined, that indicates the water deficit, and that measures vegetative and productive growth (berry weight and TLA/kg) can help elucidate how periods of water scarcity and high temperatures affect the synthesis of phenolic compounds. Prediction of the most important water stress parameters for distinguishing several sites in this study permitted a hierarchy of the five vineyards to be established. Analysis by CART has some advantages over other methods of classification or prediction for evaluating data from a pool of measurements of multiple vineyards. The first advantage is that this method is nonparametric and thus does not require any assumptions regarding the distribution of the predictors, the response or the relationship between them, and their possible interactions. Another reason for the growing popularity of this technique is its interpretability. In general, the intuitive nature of decision trees makes them simpler to interpret relative to other methods of multivariate regression. The methodology presented here can be robustly applied to large datasets to detect patterns without making any assumptions about the distribution or variance of the data. The information from these types of studies can also be useful for making better management decisions for viticulture systems. A key advantage of the tree structure is its applicability to a wide variety of variables. In the particular case of the Priorat wine growing area, due to the complex orography, the CART technique is useful to segment several varied groups of plant and grape composition data, from very hetereogeneous vineyards. This study indicates the CART technique can be used to interpret larger data sets from different crops and other areas to help interpret the physiological results obtained.

5 | MATERIALS AND METHODS

5.1 | Site location and plant material

The study was performed at five sites: two sites (Site 1 and Site 2) located in an early mesoclimate (El Molar) and three sites (Site 3, Site 4, and Site 5) in a late-ripening mesoclimate (Porrera) at different altitudes. Sites of the early region El Molar (EM) were located at: Site 1 (41°9′90″N; 0°42′75″E, elevation 100m) and Site 2 (41°9′40″N; 0°42′38″E, elevation 200m). The following three sites were selected for the late region in Porrera (PO): Site 3 (41°10′51″N; 0°52′25″E, elevation 410 m), 450 m: Site 4 (41°10′50″N, 0°52′29″E elevation 450m), and Site 5 (41°10′57″N, 0°52′32″E elevation 490 m). Carignan old bush vines were studied (50–60 years) with an average load of eight buds per vine and were planted in a density of 5000–6000 vines ha^{-1}. Vines were planted in steep terraces with a slope of 15%–25%. The soils were composed of slate conferring a stony, dry, and poor soil. Furthermore, the soils were well-drained, as they contained a high proportion (between 70% and 90%) of large particles more than 2 mm in diameter.

5.2 | Climatic characterization during vintage in both regions

Weather stations (DECAGONmodel) located in each vineyard recorded various climate data, including temperature (°C), humidity (%), rainfall (mm), and radiation (W/m^2). VPD (vapor pressure deficit) was also calculated. The early region is located near the Ebro river, is characterized by higher temperatures in summer, and lacks cool breezes. In contrast, the late region experiences sea breezes that delay maturation. Vineyards located on hillsides and terraces are drier; however, the effect of the sea breeze (i.e., *garbinada*) decreases summer temperatures, increases the relative humidity, and decreases evaporation, resulting in delayed ripening. However, the cold, dry wind that blows from the northwest along the Ebro basin (i.e., *serè*) also affects the wine growing area of Priorat. The climate of the DOCa (Denominación de Origen Calificada) is characterized by cold temperatures during the winter and hot temperatures during the summer. The annual precipitation is between 450 and 500 mm, and rains are abundant between the end of October and November.

Data that characterize climatic variation between small plots are essential for improving crop management under such extreme conditions. The weather station (Agro-climatic network in Catalonia, XAC) provided supplemental data on the weather conditions in the study area. The climate in the Priorat region (Tarragona, Spain) is characterized by high temperatures during the summer, drought, and steep poor stony soils and is thus highly vulnerable to climate change. In the early mesoclimate (El Molar, EM), the minimum temperature differences between Site 1 and Site 2 were 7°C, except in early March to mid-May and the first 3 weeks of July, where the minimum temperature differences were up to 3°C lower in Site 1. These differences, along with a slightly higher maximum temperature in Site 2, resulted in a higher thermal amplitude (AT) on the vineyard, especially from mid-May to early July and from veraison (V) to ripeness (RP) (August 15–September 21). Approximately 40% and 42% of the total precipitation, in EM (El Molar, early ripening site) and PO (Porrera, late-ripening site), respectively fell in April, and the levels of precipitation were low during the summer months. Only moderate rain values were recorded in June (20 and 19 mm in EM and PO, respectively), indicating that the summer was dry. The average temperature during the summer months was high, reaching 23.2°C in June, 25.5°C in July, and 25.8°C in August in EM and 21.4°C, 23.4°C, and 23.9°C in PO in June, July, and August, respectively.

5.3 | Phenology

The effect of climate on phenology resulted in greater variability in budbreak and veraison (V) dates depending on previous budbreak temperatures and those recorded in the spring. A temperate vintage budbreak was delayed by 8 days in Sites 3, 4, and 5 and by 11 days in Sites 1 and 2 when compared with a warm vintage. However, in a temperate vintage, the differences were less notable at the beginning of bud break (BB) and veraison (V) between the early and late regions (3 and 5 days, respectively). The high temperatures in spring resulted in an earlier fruit set in the late region, which matched the date of fruit set in the early region. Moreover, the extended summer at the end of the ripening period caused the harvest date to be 15 days prior to the normal harvest date in the region. Most earlier studies examining the effect of climate on phenology have detected reductions in the amount of time between phenological stages; however, most previous studies have been conducted in cool climate vineyards (Bock et al., 2011; Jorqueta-Fontena & Orrego-Verdugo, 2010). Date of harvest varied by 15 days between regions in the warm year, 10 days in the temperate year, and only a week in the warm year with seasonal temperature variability. A delay in bud break did not result in a delay in harvest; warm years in the late region resulted in an earlier harvest date. These observations are associated with high temperatures occurring in late August and even September in the Priorat, which results in accelerated grape ripening.

5.4 | Yields and grape ripening

Berry ripening was carefully monitored, and chemical analyses of the resulting wines were evaluated. During harvest, weekly samples of 400 berries were randomly harvested and then analyzed. Sugars (Brix), ATT (g/L total tartaric acidity), and the pH of the grape juice were determined. After crushing the whole berries, extraction of phenolic compounds was performed following a modified version of the Glories method (Nadal et al., 2010) to determine total (ANT T) and extractable anthocyanins (ANT E); %EA (extractability of Anthocyanins), %SM (seed maturity), and TPI (total polyphenol index) were also measured. OIV methods (International Organisation of Vine and Wine) were used to analyze alcohol by volume (ABV), total tartaric acidity (ATT), pH, anthocyanins, DMACA (flavan-3-ol by derivatization with *p*-dimethylaminocinnamaldehyde), and total tannins in wine. ANOVA was performed using the general linear model procedure. The Tukey test was used for post hoc analysis (XLSTAT statistical package, EXCEL) between plots.

5.5 | LWP

The LWP in each phenological stage, PS (pea size), V (veraison), and RP (ripeness), were measured using a pressure chamber (207 bar/3000 PSI pressure) (Model 600 PMS Instruments, Oaklands Park, Wokingham, United Kingdom) according to the technique described by Scholander et al., (1965). Leaf water potentials are reference measures of vine water status that have enabled solid reference thresholds of vine water status to be established. To ensure consistent readings, predawn LWP (Ψ_{PLWP}) was measured one to two hours before sunrise at 8:00 (6:00 solar time), when grapevine water status is at a maximum (Carbonneau, 1998), and midday LWP (Ψ_{MLWP}) was measured at 2:30 (12:30 solar time). In addition, primary (PLA) and secondary leaf (SLA) areas were measured during the PS, V, RP, and PH (postharvest) stages.

5.6 | Sample leaf preparation for ABA determination

Several long and tedious methods have been developed for the extraction and determination of ABA in plant tissue; however, some studies have developed more rapid approaches for the determination of phytohormones in plant material other than vine leaves (Riov et al., 1990; Setha et al., 2005). However, the establishment of a rapid method for determining ABA in vine leaves (López-Carbonell & Jáuregui., 2005), along with measurements of LWP, could provide important information for the classification of the water status of the vineyards.

Healthy leaves having reached approximately two-thirds of their definitive size were sampled from five vines per block and were bagged using Ziploc bags covered with a metalized high-density polyethylene reflective film to avoid additional leaf heating. This approach prevents the degradation of phytohormones, such as ABA. Samples were stored at −20°C. The methodology of López-Carbonell et al., (2009) was used for the extraction of ABA in Carignan leaves. Extraction solvent (Solution 1) was prepared with acetone/water/acetic acid (80:19:1, v/v/v). The solvent temperature was kept at −20°C. Reconstitution solvent (Solution 2) was prepared with water/acetonitrile/acetic acid (90:10:0.05, v/v/v). This methodology was improved by carefully weighing 4–5 g of fresh weight from a pool of different leaf samples and lyophilizing samples in a Telstar LyoQuest freeze dryer with a condenser temperature of −55°C, followed by powdering with mortar and pestle. Dried samples were carefully weighed in a 1.5-ml Eppendorf tube. Next, 1 mg of ABA internal standard was added to each of the three replicates at the beginning of the extraction procedure. A volume of 1.2 ml of extraction solvent (Solution 1) with the 300 mg of sample inside the Eppendorf was extracted in triplicate, and temperatures remained cool while samples were manipulated. The Eppendorf mixture was vortexed and left overnight at −20°C, followed by centrifugation at 15,000 ***g*** for 10 min at 4°C. Supernatants were pooled, dried under a nitrogen stream (Stuart, SBH200D), and reconstituted in 445 µl of reconstitution solvent (Solution 2), followed by stirring, vortexing, and centrifugation (10,000 ***g***, 10 min). Samples were filtered through a 0.22-µm PTFE filter (Millex Syringe-driven Filter Unit). Next, 5 ml of each sample was injected into the LC–ESI–MS/MS system. Internal standards were used for the calibration of ABA. The calibration curves for ABA showed high linearity ($R^2 = .9959$). The regression equation for the relationship between area (EIC) and ABA concentration (mg/L) was ABA = 1×10^6Area − 138.14. ABA standards were prepared daily. High correlation coefficients ($r > .995$) were obtained for concentrations ranging from 0.019 to 0.272 mg/L.

5.7 | Berry sampling and winemaking

The evolution of grape ripeness and wine composition at the five sites was followed at each of the two municipalities during the early (EM) and late (PO) mesoclimate. A total of 400 grape berries were randomly sampled. The total sugar content was measured by a refractometer. The pH was measured after homogenization of the juice. Small-scale fermentations were performed for each site in triplicate. Grapes were randomly sampled, de-stemmed, crushed into stainless-steel wine vats, and fermented after 3 days of cool maceration to extract the color and following the fermentation of all sugars. Potassium metabisulfite was added to a final concentration of 20 ppm to preserve the products of oxidation processes until bottling. The wine did not undergo malolactic fermentation. The composition of wine was determined at all five sites. Specifically, alcohol by volume (ABV), total acidity (TA), pH, total anthocyanins (Ribéreau-Gayon et al., 2000), tannins, and flavan-3-ol (DMACH method) were determined.

5.8 | HPLC analysis of anthocyanins

High-performance liquid chromatography (HPLC) was used to quantify the amount of anthocyanins and procyanidins in wines from the five treatments. Triplicates from each sample were analyzed. Anthocyanins were quantified using calibration curves of the most similar compound: malvidin-3-glucoside. Total amounts of anthocyanins were given in mg/g berry (grapes) and mg/L (wines). The different phenolic compounds analyzed were tentatively identified according to their order of elution and the retention times of pure standards (catechin, epicatechin, catechin gallate, epicatechin gallate, procyanidin B1 and B2) (Fluka). Procyanidin dimers in grape extracts were identified by analytical HPLC and comparison with authentic standards. The (−)-epicatechin *O*-gallate and B2-3′-*O*-gallate were collected from the HPLC column, and their structures were elucidated by NMR.

5.9 | Chromatographic conditions for anthocyanin analysis

Column Zorbax Eclipse Plus C18 150 × 2.1 mm, 3.5 μm (SFF-CXX, P/N 959763-902) and Precolumn Zorbax Eclipse Plus-C18 12.5 × 4.6 mm, 5 μm (SFF-C002, P/N 820950-936) were assembled over P/N 820888-901. *HPLC conditions*: injection volume 5 μl; mobile phase A Water HPLC-grade (0.2% trifluoroacetic acid); mobile phase B methanol (0.2% trifluoroacetic acid); column temperature 50°C; Detector DAD (diode array detector) (Peak width > 0.1 mm (2 s); storage of all 190–700 nm step 2 nm; slit 4 nm; margin for negative absorbance 100 mAu. *ITMS conditions*: ionization source ESI positive; ion trap analyzer (capillary 3,500 V, target mass 493 m/z, comp stability 100%, trap drive level 100%, scan 100–900 m/z, ICC smart target 500,000, max accu time 200 ms, average 5). The anthocyanidin monoglucosides of the skin extracts and wines were chromatographed by HPLC using a Beckman Ultra sphere (C18) ODS (250 × 4.6 mm i.d.) column, and detection was carried out at 520 nm. The solvents were A, H_2O/HCOOH (9:1), and B, CH_3CN/H_2O/ HCOOH (3:6:1). The gradient was 20%–85% B for 70 min, 85%–100% B for 5 min, and then isocratic for 10 min at a flow rate of 1 ml/min. The content in free anthocyanins was determined using a calibration curve (based on peak area), which was established using malvidin 3-glucoside. Standard solutions were subjected to the same procedure [concentration (mg/L) = 803.7 × (do − d) + 15.13].

The contents of free anthocyanins were determined using calibration curves (based on peak area), which were established using malvidin 3-glucoside. Standard solutions were subjected to the same procedure ($y = 0.7968x + 7.5756$, $R^2 = .9774$). The anthocyanidin-3-monoglucosides and respective acetylated and coumaroylated glycosides were identified based on their UV-Vis spectra and retention times. The anthocyanidins were identified by HPLC by comparison with internal standards. The calibration curves were obtained by injecting standards with different concentrations of malvidin 3-glucoside (Sigma). The range of linear calibration curves was from 0.1 to 1.0 mg/L for the lower concentration compounds ($R^2 > .996$), 0.1 to 5.0 mg/L for intermediate concentration compounds ($R^2 > .987$), and 10.0 to 200.0 mg/L for the higher concentration compounds ($R^2 > .987$). Unknown concentrations were determined from the regression equations, and the results were expressed in mg of malvidin 3-glucoside per berry. Repeatability of this method from extraction to HPLC analysis for four samples of the same batch of grape skins had a coefficient of variation <7%.

5.10 | Statistics

The water potential, leaf temperature, and grape and wine composition were evaluated through one-way ANOVA, and when $p < .05$, Tukey post hoc tests were used. A Pearson correlation matrix was calculated for all parameters with a significance level (α) of 0.05.

CART (classification and regression trees) analysis was performed using XLSTAT (Microsoft Excel statistical add-in). The decision tree method is a powerful and popular predictive machine-learning technique that is used for both classification and regression (Breiman et al., 1984). Thus, the methods are also known as Classification and Regression Trees (CART). The algorithm of decision tree models repeatedly partitions the data into multiple subspaces, so that the outcomes in each final subspace are as homogeneous as possible. Among all measured variables, the CART technique acts as a predictive model that shows the more significant variables to distinguish each final subspace. The tree models predict the outcome by asking a set of *if–else* questions. Regression tree analysis predicted the outcome as a real number (leaf temperature and water potential). The start of the tree was at the root node; for each variable, CART finds the set that minimizes the sum of the node impurities in the two child nodes and chooses the split that gives the minimum overall variable and set. The measure of the node impurity is based on the distribution of the observed values in the node; splitting stops if the relative decrease in impurity is below a pre-specified threshold.

ACKNOWLEDGMENTS

We would like to aknowledge the University Rovira i Virgili, Biochemistry Department, Viticulture Research Group. Partial support for this work has been received from the Agency for Management of University and Research Grants (AGAUR) of the Government of Catalonia (grant 2017 SGR 705). Also we would like to acknowledge the anonymous reviewers for helping to improve the manuscript and supporting information.

AUTHOR CONTRIBUTIONS

J.M.M designed the statistical data analysis A.S performed the experiments. M.N supervised the water stress experiments, and M.L provided technical vineyard data assistance A.S. agreed to serve as the author responsible for content and communication.

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How to cite this article: Sánchez-Ortiz A, Mateo-Sanz JM, Nadal M, Lampreave M. Water stress assessment on grapevines by using classification and regression trees. *Plant Direct*. 2021;5:e00319. https://doi.org/10.1002/pld3.319