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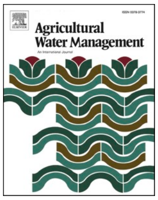

Phloem carbon isotopic signature as a valuable tool to assess physiological adjustments among European grapevine varieties under a Mediterranean climate

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ABSTRACT

Wine production is being threatened by climate change, particularly in the Mediterranean region. Thus, more knowledge on vineyards' well-functioning and adjustments to drought conditions is essential. We aimed to understand how grapevine varieties from different European origins, under the same climatic context, respond to water stress and whether the climatic origin plays a role in their water-use efficiency (WUE). With that in mind, we assessed the carbon isotopic signatures ($\delta^{13}C$) in berry juice and phloem of 172 white and red grapevine varieties grown in Herdade do Esporão (Alentejo, Portugal) from five different climatic origins in two different rainfed water treatments: with full and no irrigation. Our findings indicate that there is a ^{13}C enrichment in both berry juice and phloem under water stress, regardless of the climatic origin of the grapevine varieties. This suggests that the plants exhibited greater stomatal regulation and tighter control over water loss during drought conditions. Also, we found a positive correlation between berry juice and phloem $\delta^{13}C$ signatures that is maintained under limited water conditions (i.e., non-irrigated rainfed condition: NI-rf). Six clusters, based on $\delta^{13}C$ patterns in NI-rf, were obtained and the varieties that showed the higher WUE listed. The patterns of $\delta^{13}C$ drought-adjustments (differences among water-treatments) were dependent on specific varieties capacity to acclimate, and the varieties that were grouped as the ones with high WUE in NI-rf also showed greater $\delta^{13}C$ drought-adjustments. These results highlight a range of WUE adjustments, revealed through phloem isotopic analysis, being phloem $\delta^{13}C$ signature a complementary and promising proxy to assess grapevine varieties' drought acclimation capacity. Our study reinforces vineyard plasticity and great adaptative ability, transversal to several grapevine varieties with different climatic origins. The results provided might be a complementary support to identify the varieties that are potentially more physiologically equipped under drought, and to further explore their vitality and grape quality under sustainable water-management scenarios.

1. Introduction

Wine production has great value worldwide, being one of the main agro-industrial activities (Rockenbach et al., 2011), involving several producing countries, encompassing distinct climatic conditions (Hussain et al., 2008; OIV, 2019). With 40 % of the vineyard (*Vitis vinifera* L.) area accounted in the Mediterranean region (FAOSTAT, 2018), wine production is being undoubtedly challenged by climate change with a widespread dryness ascribed to reduced precipitation and higher temperatures (IPCC, 2022). These worrying scenarios will threaten vineyards well-functioning since the whole-system dynamics highly depends on biotic and abiotic interactions to be as functional as profitable (Burkhard and Maes, 2017). Climate fluctuations, particularly drought and heat waves, affect vineyard phenology by altering the timing of growing season and, consequently, the optimal phase for maturation. Moreover, grapevine exhibit a wide range of traits that frames physiological strategies, which defines how grapevines respond to drier conditions (Wolkovich et al., 2018). It is therefore expected that different varieties will perform differently under drier conditions.

Different geographic origins lead to genetic adaptations associated

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with experienced contrasting climate conditions during their evolution process (Pouzoulet et al., 2020). Therefore, resistance to drought (soil and atmosphere) could be highly variable considering each variety response. Vineyard efficiently responds to water stress through many different strategies, such as highly deep and well-developed root system to maximize water uptake (Bauerle et al., 2008), stomata regulation that controls transpiration (Charrier et al., 2018), as well as regulatory mechanisms within xylem to avoid embolisms (Jones and Sutherland, 1991; Pouzoulet et al., 2020). Linked to this, in situ varietal selection could provide varieties better adapted to new climatic scenarios (Morales-Castilla et al., 2020). Indeed, winemakers from northern Europe are selecting varieties from a range of southern Europe (although limited to varieties adapted to warm and dry climates) (Stock et al., 2005; Santos et al., 2020).

To mitigate the impacts of water scarcity, most of the Mediterranean vineyards are already relying on irrigation during drought periods (Zarrouk et al., 2016). Since water resources are decreasing by unsustainable practices (AQUASTAT-FAO, 2013; Engel et al., 2015; Costa et al., 2020), more sustainable strategies are needed. It has been implemented in the last decades, a deficit and precision irrigation, reducing water use from irrigation, while maintaining yield and wine quality, by improving the yield to water consumption ratio, and thus increasing water use efficiency (WUE) (i.e., instantaneous WUE, the ratio of carbon assimilated by photosynthesis to the water lost in transpiration (A/E), or intrinsic WUE, the ratio between carbon dioxide assimilation and stomatal conductance (A/gs)) (Farquhar and Richards, 1984; Farquhar et al., 1989; Seibt et al., 2008; Flexas et al., 2010; Medrano et al., 2015). The watering strategies applied in vineyards aim to improve berry characteristics considering proper seasonal ripening (Chaves et al., 2007; Zarrouk et al., 2012; Bchir et al., 2016) without compromising vineyard longevity (Costa et al., 2020). Considering predicted scenarios of drought and water scarcity, it is essential to know how a large set of grapevine varieties change their WUE when only rainfed under a Mediterranean climate.

Ecophysiological studies under different irrigation conditions, including the absence of artificial watering, can contribute to understanding vineyard water status, their WUE, and processes involved in grapevine responses to water deficit (Choné et al., 2001; Gaudillère et al., 2002). Several studies relied on stable isotopic analysis to infer water-use strategies and efficiency in vineyards (e.g., Gibberd et al., 2001; Herrero-Langreo et al., 2013; Santesteban et al., 2015), using δ^{13}C grape juice and must carbon isotopic signature (δ^{13}C) as a proxy for vine water status and WUE (Gaudillère et al., 2002; Deloire et al., 2005; Herrero-Langreo et al., 2013; Brillante et al., 2020). Also, sugar δ^{13}C in mature berries integrates photosynthetic isotopic discrimination during berry ripening, which can be linked to soil water availability (Gaudillère et al., 2002; Santesteban et al., 2015). Under drought conditions, these processes could be compromised: while moderate water stress could favor sugars redistribution to berries fostering quality wines, severe water stress constrains phloem unloading through sugars' accumulation inhibition (Patrick, 1997; Carbonneau and Deloire, 2001). Water stress will increase δ^{13}C (Bota et al., 2001; Costa et al., 2012; Lovisolo et al., 2016), and grapevine varieties are expected to show different sensitivities to water deficits, reflected in ^{13}C enrichment. When the ratio of intercellular to ambient CO_2 concentration (ci/ca) is reduced (due to either stomatal closure or increased photosynthesis rates), there is less discrimination against ^{13}C resulting in more enriched δ^{13}C signatures and thus higher WUE (Farquhar et al., 1989). Moreover, δ^{13}C might be expressed differently across different grapevine components and integrate different time-scales (Souza et al., 2005b; Seibt et al., 2008; Flexas et al., 2010). Grapes are an extremely efficient carbon sink, integrating photosynthetic conditions during all the ripening period (the time sugars in the berry juice accumulate), and are considered a suitable long-term indicator of plant WUE in some grapevine varieties (Bota et al., 2004; Flexas et al., 2010; Santesteban et al., 2015; Bchir et al., 2016). Whereas δ^{13}C of water-soluble organic matter in phloem contain recently assimilated carbon and is expected to respond more dynamically to (short-term) environmental conditions and species characteristics controlling photosynthetic discrimination (Scartazza et al., 2004; Gessler et al., 2008; Rascher et al., 2010). Phloem sap δ^{13}C has the potential to be an integrative tracer of changes at whole canopy level and a tool for estimating canopy-level carbon assimilation rates and whole plant stomatal conductance (Keitel et al., 2006; Rascher et al., 2010). Yet, studies are particularly lacking on grapevine phloem, and across a wide range of varieties under the same climate.

Thus, it is essential to have a better knowledge of the effects of drought on grapevine physiology, particularly WUE, and explore new proxies that would help to reveal drought-adapted varieties. In this context, we aim to understand (i) the patterns of δ^{13}C (reflecting their WUE) of European grapevine varieties, in non-irrigated (rainfed) conditions under a Mediterranean climatic context, (ii) whether varieties' climatic origin plays a role on their δ^{13}C signatures and drought adjustments, (iii) if there is a relationship between δ^{13}C in berry juice and phloem, and if the latter can be a complementary tool to discriminate varieties with high WUE (and potentially better drought adapted).

2. Materials and methods

2.1. Ampelographic field and experimental design

The study was conducted in the Ampelographic Field of Herdade do Esporão (CAHE) in Reguengos de Monsaraz, Alentejo, Portugal (38.380098ºN, 7.560724ºW). This field has a collection of 189 grapevine varieties (*V. vinifera*) planted in 2011, including 87 red varieties, 98 white varieties, and four rose varieties, from several geographic origins. From these 189 grapevine varieties, 172 were selected (Supporting Tab. S1).

The field site is characterized by a temperate climate with hot and dry summers, classified as 'Csa' according to Köppen-Geiger Climate Classification (IPMA, 2021a). Historically, the annual average temperature is 16,1 °C, and the annual precipitation is 572 mm (CRU-TS 4.03 (Harris et al., 2014) downscaled with WorldClim 2.1 (Fick and Hijmans, 2017). The seven-year-old grapevines, grafted on 1103 P, were spaced 3 m between and 1,5 m along North-South oriented rows and trained on a vertical shoot positioned system. The soil is a Eutric Cambisol with a ApBw1Bw2C profile, derived from granite with 75–80 % of sand.

Grapevine varieties were submitted to two irrigation treatments:

i) full irrigation (hereafter classified as FI) - irrigation was applied weekly, with the irrigation amount determined as: $I = ETo * Kcb * 1.1$ (Allen et al., 1998 equation adapted to consider local soil water evaporation). Daily ETo (evapotranspiration) was calculated from meteorological data measured locally using the Penman-Monteith FAO 56 method (Allen et al., 1998), and the Kcb (crop coefficient) was estimated using the formula $Kcb = 1{,}44 * NDVI - 0{,}1$ (Campos et al., 2010). Normalized Difference Vegetation Index (NDVI) was extracted from Sentinel-2 imagery along the vegetative cycle (from the beginning of May until September of 2018). The 1.1 factor was used to consider soil water evaporation, based on previous measurements with microlysimeters (obtained at a vineyard located at the same region and with the same irrigation frequency; unpublished data). ETo, Kc and I data are presented in Suplementary Figs. S1 and S2.

ii) non-irrigated rain-fed (from now on classified as NI-rf) – no irrigation applied, grapevines only relying on rain (precipitation amount is shown in Fig S2).

2.2. Field sampling

In 2018, phloem and grape samples were collected from the 172 grapevine varieties under the two studied treatments, in three plants from each variety ($n = 516$ per treatment). Monthly mean temperatures ranged from 36 to 14 ºC, recorded in August and January, respectively, while mean precipitation was 100 mm during winter and only 31 mm in summer (IPMA, 2021b). The sampling season was particularly dry, with a total rainfall during summer of only 35 % comparing to the long-term average, including a drastic heat wave occurring in summer. In August, was observed a positive anomaly of 4.3 ºC in the average temperature and of 7 ºC in the maximum daily temperatures (comparing to the long-term period of 1981–2010) (IPMA, 2021a).

Phloem was collected in August, by cutting 3–4 small rectangles of stem bark (superficial portions of the stem of ca. 2 cm^2 avoiding xylem collection) with a razor blade, at the middle of the stem (between the 5th and the 7th node), from 1-year-old twigs, and placed in a sterile tube containing 2 ml of ddH2O, and left to rest at least 5 h at room temperature (Gessler et al., 2004; Scartazza et al., 2004; Rascher et al., 2010). To minimize differences caused by the daytime period and inter-day variability, the sampling was performed by a large team (around 40 persons previously trained), during the afternoon, in two consecutive days with similar weather conditions.

Grapes were collected at technological maturation, which was evaluated by berry refractometry according to the varieties' precocity (°Brix), and taking into account the main phenological stages observed in 2018 and the number of days between flowering and maturation (based on a historical dataset of 6 years). Thus, grape sampling was done in seven phases according to the timming at which maturation was reached by the varieties. Approximately six berries from three clusters per vine (ca. 18 berries per plant) were collected and stored in falcon tubes. All phloem ($n = 1032$) and berries ($n = 1032$) samples were frozen until further analysis.

2.3. Carbon isotopic composition analysis

Phloem samples were thawed at room temperature and homogenized in the vortex. A volume of 200 μL of soluble sugars from the phloem samples (phloem exudates) was transferred to a square thin capsule and dried at 60 °C for at least 48 h (to evaporate the liquid) (Gessler et al., 2004; Rascher et al., 2010).

Before analysing berries from all the varieties, seven varieties were randomly selected to test two methods: (i) by removing the peal from the grape pulp, followed by macerating the pulp and retrieving the juice and (ii) by pressing berries to get the juice from the compost sample. A volume of 30 μL of berry juice (resulting from each method) was transferred to tin weighing capsules and dried at 60 °C for at least 48 h. In total 84 samples were used to sought the correlation between the two methods (Supporting Fig. S3). Due to the high correlation between the two methods (Pearson $r = 0.84$, $p < 0.001$), the most effortless method was used for all berry samples., i.e., method (ii).

All samples were encapsulated and analysed for carbon isotopic composition ($\delta^{13}C$) determined by continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Sercon Hydra 20–22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser, at the Stable Isotopes and Instrumental Analysis Facility (SIIAF - FCUL). Uncertainty of the isotope ratio analysis, calculated using values from 6 to 9 replicates of secondary isotopic reference material interspersed among samples in every batch analysis, was $\leq 0.1‰$.

In this study, leaf $\delta^{13}C$ was not considered since the effect of irrigation and drought on $\delta^{13}C$ is expected to be more pronounced in grape berries than in leaves, as postulated by Bchir et al. (2016), highlighting berry $\delta^{13}C$ as the best indicator of plant water status. Also, $\delta^{13}C$ of pulp showed a better correlation with intrinsic water use efficiency (the ratio between carbon dioxide assimilation and stomatal conductance, A/gs) and water potential than grapevine leaves (Souza et al., 2005b).

2.4. Data analysis

The 172 grapevine varieties were classified according to their climatic origin by first specifying their country of origin (based on Robinson et al., 2013 and Cunha et al., 2016) and then assigning a Köppen-Geiger climate classification (based on maps of Köppen, 1936) (such as Bsk - Arid, step, cold; Csa – temperate, dry summer, hot summer; Csb – temperate, dry summer, warm summer; Cf – temperate, no dry season; Dfb – Cold, no dry summer, warm summer) (Supplementary table S1). A cluster analysis were performed based on grape and phloem $\delta^{13}C$ data from the non-irrigated rain-fed trateament (NI-rf), using Euclidean distance method, and kmeans function in R (R Core Team, 2022). After evaluation of within sum of squares results, by selecting the number of cluster (k) where reduction in the total weighted sum of squares begins to flatten, 6 clusters were considered ($k = 6$). We then evaluated if the clusters obtained agreed well with the climatic origins classes and/or the color of the grape.

To seek relations between berry juice and phloem $\delta^{13}C$, Pearson correlations ($\alpha = 0.05$) were performed on the total data ($n = 172$) and Spearman correlations performed for each cluster group previously defined (due to lack of normality in most of the cluster groups), considering the two irrigation treatments separately (using *Hmisc* R package (Harrell, 2022).

The differences of $\delta^{13}C$ between treatments (i.e., the difference between mean $\delta^{13}C$ of NI-rf and FI in each variety), for both berry and phloem plant components, were calculated. The effect of 'climatic origin' on this FI to NI-rf $\delta^{13}C$ differences was tested through a non-parametric Kruskal-Wallis test, at a significance level of $\alpha = 0.05$ (using *agricolae* R package (de Mendiburu and de Mendiburu, 2019). Since we had an extremely unbalanced dataset regarding climatic origins, we compared our results with a test applied to a transformed dataset (i.e., with a combination of over- and under-sampling: balancing the dataset by increasing the size of the 'rare' samples through simple repetition, and by reducing the size of the abundant classes through the random selection of 15 samples on each variety that had more than 15 samples); and the outcomes were the same, supporting the results.

The effect of 'cluster' (previously generated) on the FI to NI-rf $\delta^{13}C$ differences, and the differences among clusters, were evaluated through an ANOVA (the assumptions of homoscedasticity and normality were met), followed by a Tukey post hoc test (using R functions from'stats').

All statistical analyses and plotting were performed using R Statistical software (R Core Team, 2022).

3. Results

The cluster analysis based on the patterns of $\delta^{13}C$ of the studied grapevine varieties in non-irrigated rainfed (NI-rf) conditions revealed six groups (Fig. 1, Table 1), which were not in accordance with either their climatic origin or grape color (Fig. S4). Cluster 1 and 2, which encompassed 17 and 26 varieties respectively (Table 1), represented the varieties with the more enriched values of ^{13}C (in both phloem and grape) under water stress (Fig. 1).

By exploring the relationship between carbon isotopic signature in berry juice and phloem, we found a general positive significant correlation (Fig. 2). This positive relationship is observed in both FI and NI-rf, with no major effect of water stress in the relationship between the two plant components analysed. Neverthless, in the NI-rf treatment the clusters (groups of varieties) showed negative and positive correlations between the berry and phloem $\delta^{13}C$.

Our results showed that regardless of grapevine varieties' climatic origin, there was a common ^{13}C enrichment under water stress for berry juice and phloem $\delta^{13}C$ (Fig. 3, Fig. S5). There were no significant differences among climatic origins (Fig. S5), and the most enriched values, for both plant components, were mostly belonging to clusters 1 and 2 (Fig. 4). Varieties with higher values of grape and phloem $\delta^{13}C$ under drought (clusters 1 and 2 – Fig. 1 and Table 1), were also the ones showing higher differences of $\delta^{13}C$ between water treatments, i.e., drought adjustments; while the ones with the most depleted values of $\delta^{13}C$ (cluster 6 – Fig. 1 and Table 1), were the ones showing lower $\delta^{13}C$ differences between water treatments (Fig. 4). The decreasing differences between FI and NI-rf were more clear in phloem than in grape

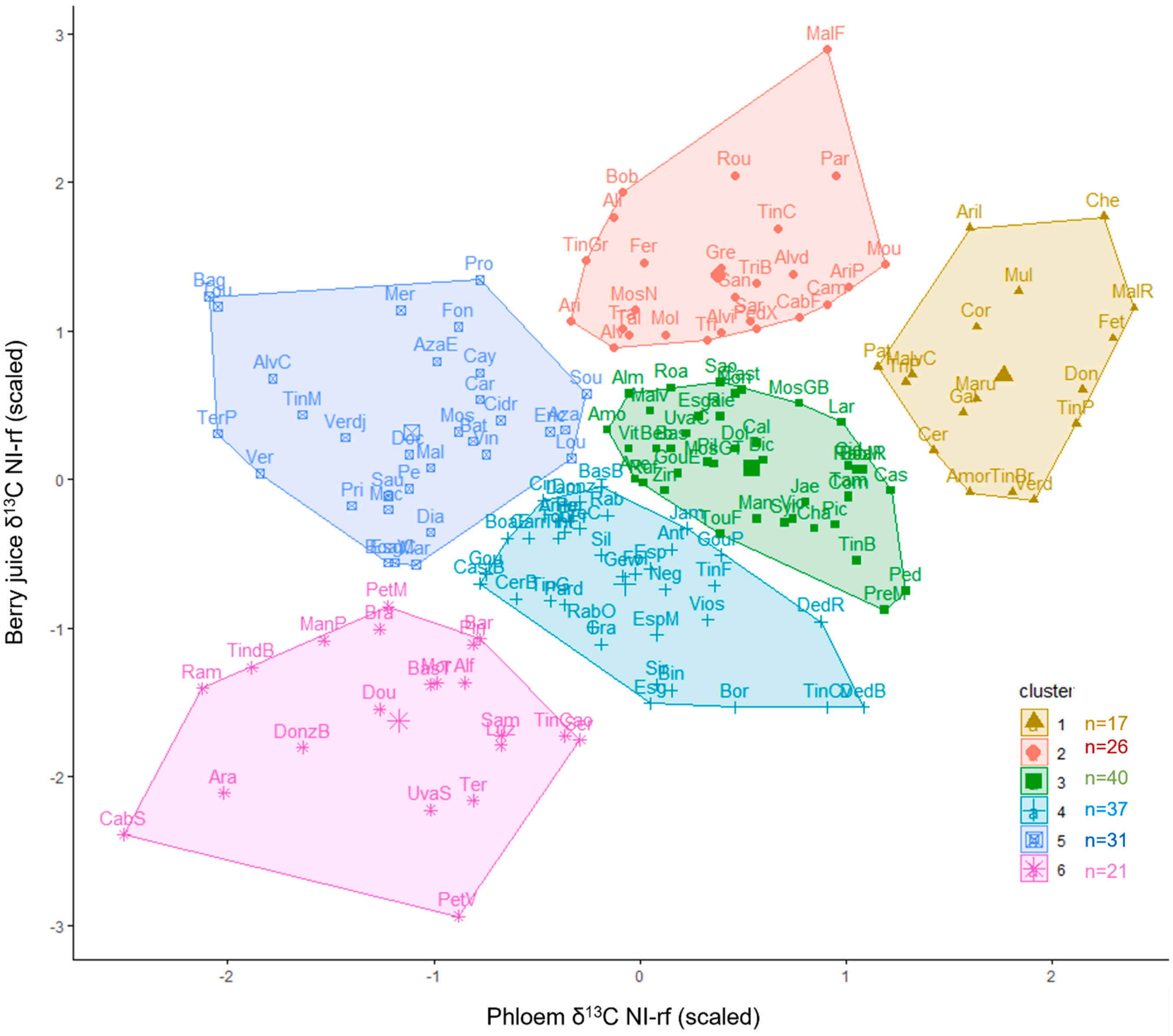

Fig. 1. – Grapevine varieties (n = 172) assigned to clusters based on berry juice and phloem carbon isotope composition ($\delta^{13}C$) under no irrigation rain-fed (NI-rf) conditions (see varieties names in Table 1).

(Fig. 4). Additionally, although the range of $\delta^{13}C$ in NI-rf was different among clusters, their isotopic signatures in FI were similar in the grape component (Fig. S6). While considering the phloem $\delta^{13}C$ at FI, cluster 1 segregated from the others, showing a distinct enriched value even in FI Fig. S6.

They all shared a common negative correlation between $\delta^{13}C$ in FI and the differences between NI-rf and FI (drought adjustments) in both phloem and grape (Fig. S7). In both components, with increasing $\delta^{13}C$ in FI conditions, a lower difference is denoted between water treatments in all groups; in other words, within their cluster range, higher isotopic adjustments are observed when lower $\delta^{13}C$ is experienced under full irrigation (Fig. S7).

4. Discussion

By exploring the $\delta^{13}C$ signatures of European grapevine varieties growing in non-irrigated rainfed conditions (NI-rf) under a Mediterranean climatic context, we delimited different groups/clusters of varieties, reflecting their stomatal control and water-use-efficiency (WUE) under drought. We found that the climatic origin did not played a role on these isotopic signatures' groups, neither on the water treatments differences observed.

Both berry juice and phloem showed an enrichment in ^{13}C under water stress, regardless of their climatic origin. We also found different ranges of $\delta^{13}C$ enrichment under NI-rf across grapevine varieties. A higher water stress and needed acclimation, was translated into higher stomatal adjustments (Sperry, 1986; Sperry et al., 2002; see Gambetta et al., 2020 and references within), and particularly for a group of specific varieties (identified in Table 1 as clusters 1 and 2). Under drought, stomatal regulation and leaf gas exchange are processes highly determinant for water stress resilience (Bota et al., 2001; Palliotti et al., 2009). This behavior would allow the varieties making the higher stomatal adjustments and showing higher $\delta^{13}C$ in both grape and phloem, and therefore higher WUE (i.e., the varieties from clusters 1 and 2, Fig. 1 and listed in Table 1) to be potentially more physiologically aquipted under drought.

There is a range of ^{13}C enrichment that can be supported by genotypes ascribing differential vineyard responses to drought (Chaves et al.,

Table 1
Grapevine varieties (name and code, grape color: R=red, W=white), grouped by $\delta^{13}C$ patterns (see clusters in Fig. 1).

Cluster 1 and 2		Cluster 3		Cluster 4		Cluster 5 and 6	
Variety	**Code**	**Variety**	**Code**	**Variety**	**Code**	**Variety**	**Code**
Amor-Não-Me-Deixes R	Amor	Almafra W	Alm	Amaral R	Ama	Alvarelhão Ceitão R	AlvC
Arinto do Interior W	AriI	Amostrinha R	Amo	Antão Vaz W	Ant	Azal W	Aza
Cercial W	Cer	Avesso W	Ave	Bastardo Branco W	BasB	Azal Espanhol R	AzaE
Chenin W	Che	Bastardo R	Bas	Binzelo W	Bin	Baga R	Bag
Corropio N	Cor	Beba W	Beb	Boal Espinho W	BoaE	Batoca W	Bat
Dona Branca W	Don	Bical W	Bic	Borraçal R	Bor	Boal Vencedor W	BoaV
Feteasca Alba W	Fet	Boal Ratinho W	BoaR	Carrasquenho R	Carr	Carignan R	Car
Galego Dourado W	Gal	Caladoc R	Cal	Castelão Branco W	CastB	Cayetana W	Cay
Malvasia Rei W	MalR	Cascal W	Cas	Cerceal Branco W	CerB	Cidreiro R	Cidr
Malvasia Cândida W	MalvC	Castelão R	Cast	Cinsaut R	Cin	Diagalves W	Dia
Marufo R	Maru	Chacelas W	Cha	Dedo de Dama W	DedB	Doçal W	Doc
Muller Thurgau W	Mul	Cidadelhe R	Cid	Dedo de Dama R	DedR	Encruzado W	Enc
Patorra R	Pat	Cornifesto R	Corn	Donzelinho R	DonzT	Esgana Cão W	EsgC
Tinta Bragão R	TinBr	Dolcetto R	Dol	Esganinho W	Esg	Fonte Cal W	Fon
Tinta Pomar R	TinP	Esganoso W	Esga	Espadeiro R	Esp	Loureiro W	Lou
Trincadeira das Pratas W	TriP	Gouveio Estimado W	GouE	Espadeiro Mole R	EspM	Macabeo W	Mac
Verdelho W	Verd	Jaen R	Jae	Folgasão W	Fol	Malbec R	Mal
		Larião W	Lar	Gewurtztraminer W	Gew	Marselan R	Mar
Alicante Bouschet R	Ali	Malvasia W	Malv	Gouveio W	Gou	Merlot R	Mer
Alvarelhão R	Alv	Manteúdo W	Man	Gouveio Preto R	GouP	Moscatel Graúdo W	Mos
Alvadurão W	Alvd	Monvedro R	Mon	Granho W	Gra	Pé Comprido W	Pe
Alvarinho W	Alvi	Moscatel Galego W	MosGB	Jampal W	Jam	Prieto Picudo R	Pri
Arinto W	Ari	Moscatel Galego R	MosGT	Lameiro W	Lam	Prosecco W	Pro
Arinto do Pico W	AriP	Pedral R	Ped	Negra Mole R	Neg	Sauvignon W	Sau
Bobal R	Bob	Pical R	Pic	Pardina W	Pard	Sousão R	Sou
Cabernet Franc R	CabF	Pilongo R	Pil	Perrum W	Per	Terrantez do Pico W	TerP
Camarate R	Cam	Preto Martinho R	PreM	Preto Cardana R	PreC	Tinta Miúda R	TinM
Fernão Pires W	Fer	Rabigato Moreno W	RabM	Rabigato W	Rab	Touriga Nacional R	Tou
Grenache R	Gre	Riesling W	Rie	Rabo de Ovelha W	RabO	Vermentino W	Ver
Malvasia Fina W	MalF	Roal R	Roa	Silvaner W	Sil	Verdejo W	Verdj
Molar R	Mol	Rufete R	Ruf	Síria W	Sir	Vinhão R	Vin
Moscatel Nunes W	MosN	São Mamede W	Sao	Tinta Carvalha R	TinCv	Alfrocheiro R	Alf
Mourisco Branco W	Mou	Syrah R	Syr	Tinta Francisca R	TinF	Aragonez R	Ara
Parellada W	Par	Tamarez W	Tam	Tinta Gorda R	TinG	Barcelo W	Bar
Pedro Ximenez W	PedX	Tinta Barroca R	TinB	Tintinha R	Tint	Bastardo Tinto R	BasT
Roupeiro Branco W	Rou	Touriga Fêmea R	TouF	Touriga Franca R	TouFr	Branjo R	Bra
Sangiovese R	San	Uva Cão W	UvaC	Viosinho W	Vios	Cabernet Sauvignon R	CabS
Sarigo W	Sar	Viognier W	Vio			Donzelinho Branco W	DonzB
Tália W	Tal	Vital W	Vit			Douradinha W	Dou
Tinta Caiada R	TinC	Zinfandel R	Zin			Luzidío W	Luz
Tinta Grossa R	TinGr					Manteúdo Preto R	ManP
Trajadura W	Tra					Moreto R	Mor
Trincadeira R	Tri					Petit Maseng W	PetM
Trincadeira Branca W	TriB					Petit Verdot R	PetV
						Pinot Noir R	Pin
						Ramisco R	Ram
						Samarrinho W	Sam
						Sercial W	Ser
						Terrantez W	Ter
						Tinto Cão R	TinCao
						Tinta da Barca R	TindB
						Uva Salsa W	UvaS

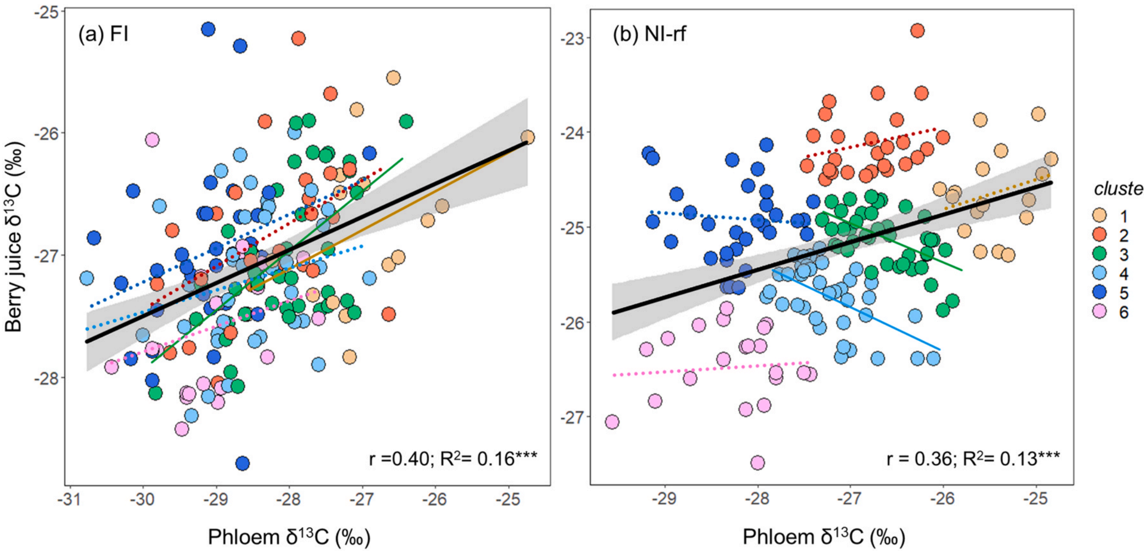

Fig. 2. – Correlations between carbon isotopic composition ($\delta^{13}C$, ‰) of berry juice and phloem for 172 different grapevine varieties, subjected to two water treatments: (a) Full irrigated (FI) and (b) Non-irrigated rainfed (NI-rf). Linear fit (with full lines when statistically significant and a dashed line when the trend was not statistically significant) for all varieties (black line, and Pearson coefficient of correlation r and coefficient of determination R^2) and for each group (colored lines). Note that y-axis has different scales in (a) and (b).

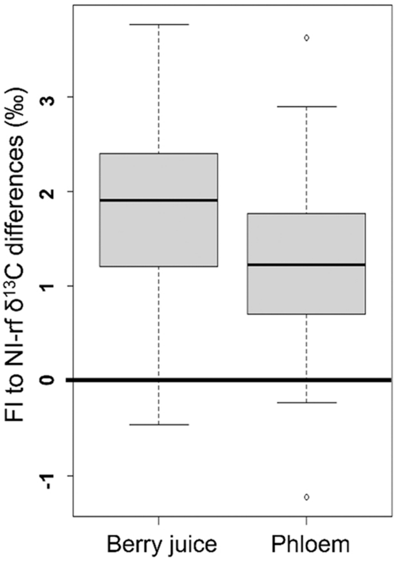

Fig. 3. – Boxplots of the differences between the two water treatments: full irrigation (FI) and no-irrigation rain rainfed conditions (NI-rf), considering the carbon isotopic composition ($\delta^{13}C$, ‰) of berry juice and phloem for the total grapevine varieties studied (n = 172). Zero represents no differences between the two water treatments, and positive values higher $\delta^{13}C$ in NI-rf than FI.

1987; Bota et al., 2001; Palliotti et al., 2009). The degree of stomatal conductance seems to depend on each variety's specific acclimation capacities, as already proposed by Chaves et al. (2010). The shift under drought towards an increased WUE, due to the above-mentioned increased stomatal control and reduced transpiration, are expected in deficit irrigation and water-stressed conditions (Souza et al., 2005a; Souza et al., 2005b; Gómez-Alonso and García-Romero, 2009; Zufferey et al., 2017). Studies carried out in different grapevine varieties and in different locations (e.g., Stoll et al., 2000; Loveys et al., 2004; Poni et al., 2007; Marsal et al., 2008) reinforce the ubiquity of stomatal control as a strategy to deal with drought in grapevines and their inherent plasticity. In other words, although stomatal control is ubiquitous among grapevine varieties (Fig. 3), there is a differentiation of the degree of stomatal adjustments (and WUE) among the varieties (Fig. 4). The greater physiological adjustments, supporting a higher WUE, might eventually promote or affect plant vigor, vitality and grape quality. Thus, although not included in this study, it is of great importance to further study other physiological aspects and grape quality to completely understand the most drought-adapted varieties.

According to our results, phloem might be valuable for assessing plant varieties' responses under drought, contributing to show isotopic patterns of similarity (and dissimilarity) among varieties under drought (i.e in NI-rf). Since it is the plant tissue that will reflect recently fixed carbon (e.g., 1–3 days' time lag), changes in the environment such as drought will interfere more directly with phloem $\delta^{13}C$ (with less stored and reused carbon counfounding factors) than berry juice (which will consist of a more complex mixture of carbon with different turnover times and metabolic history) (Scartazza et al., 2004, 2015; Keitel et al., 2006; Gessler et al., 2008). Thus, the phloem ^{13}C signature is expected to reflect the conditions of the sampling moment, whereas the berry signature will integrate conditions from a wider time range (from fruits' formation to maturation). This is particularly relevant under NI-rf since phloem $\delta^{13}C$ highlighted for adjustments in certain varieties that otherwise would be missed if only berry juice $\delta^{13}C$ was assessed. Albeit the common ^{13}C enrichment from FI to NI, and the expected ranges in grape and phloem C-isotope composition (because they correspond to different plant organs subjected to different metabolic processes and ^{13}C fractionation), we were able to find that carbon isotopic signature in phloem generally reflects $\delta^{13}C$ of berry juice (reflecting plant WUE) (Fig. 2). Furthermore, this general positive relationship is maintained under the drought conditions of NI-rf treatment, and the positive trend kept in the most ^{13}C enriched varieties (from clusters 1 and 2). The different type of correlations at the variety/cluster level, reflects the complexity of carbon allocation, use and partitioning along different periods of time. The phloem to grape positive relationship is thus more evident in the group of varieties with higher $\delta^{13}C$ values (indicating higher WUE at both short- and long-term), and are decoupled or negative in the lower or intermediate ranges of $\delta^{13}C$. Within these clusters' isotopic ranges, varieties that show short-term drought adjustments towards higher intrinsic WUE, are the ones that show lower WUE through long-term indicators (i.e., berry juice $\delta^{13}C$), possibly through the maintenance of a higher gs and/or lower A along the ripening period for these varieties that are still able to promptly lower their gs and/or

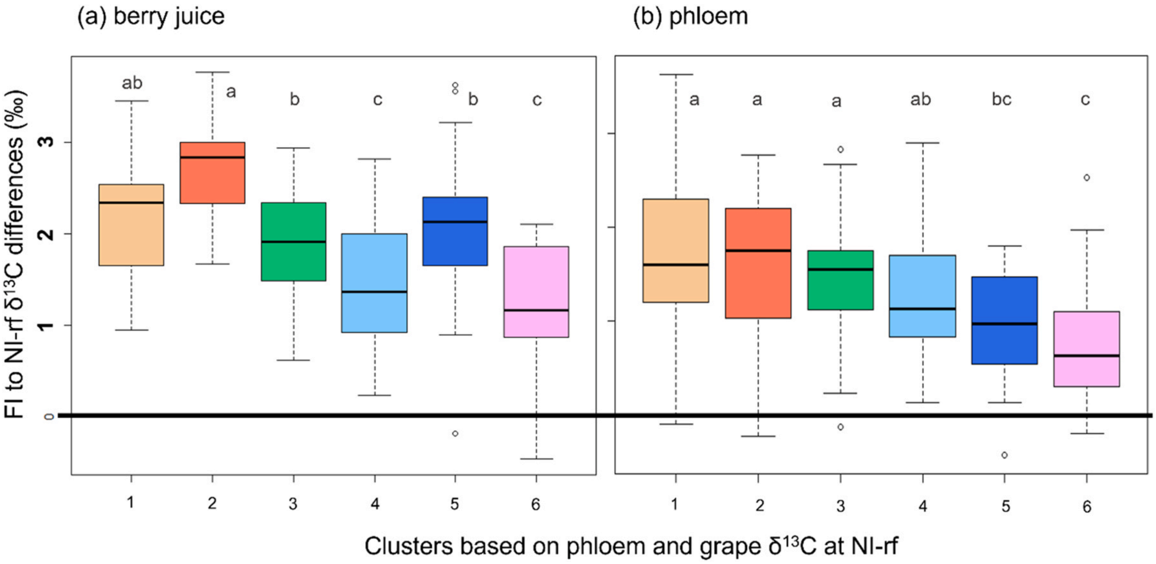

Fig. 4. – Boxplots of the differences between the two water treatments: full irrigation (FI) and no-irrigation rain rainfed conditions (NI-rf), considering the carbon isotopic composition ($\delta^{13}C$, ‰) of berry juice and phloem for the 6 clusters generated (see Fig. 1). Different letters represent significant differences ($p < 0.05$) among clusters and open dots the outliers.

increase A under drought-stress. Nevertheless, the combined analysis of carbon isotopes in phloem and berry juice could be very useful to characterize the WUE adjustments of grapevines under NI-rf conditions, and identify those varieties that show both short- and long-term increased WUE under drought. Thus, the ability to detect particular WUE short-term adjustments while integrating longer-term carbon assimilation patterns, makes phloem $\delta^{13}C$ a tool to consider when assessing drought responses in specific grapevine varieties.

Considering the importance of grapevine response to drought, our study addressed the relevance of phloem isotopic signature to assess grapevine varieties' acclimation capacity. This can be useful knowledge on the emergent topic of in situ varietal selection to choose better-adapted varieties (reviewed by Mosedale et al., 2016). *V. vinifera* varieties domesticated in semi-arid conditions are generally vulnerable to drought (Pouzoulet et al., 2020), yet they do show different ranges of WUE under drought. Assessing their WUE in different time-scales (short and long-term responses under drought) through grape and phloem $\delta^{13}C$ can be the window of opportunity for detecting better drought-adaptations of wine varieties, particularly in Mediterranean-type climate. In this sense, it would be of great interest to infer WUE in a larger time span through phloem $\delta^{13}C$, by sampling throughout various time points considering the grape maturation continuum, and accompanying berry development, and eventually implement the best management techniques in the most efficient timings aiming sustainability.

5. Conclusions

By studying a large number of grapevine varieties (172), under a Mediterranean climate and two water treatments, we showed the great physiological plasticity of wine varieties. This plasticity may allow grapevines to maintain productivity and quality while under sustainable water management (no artificial irrigation), particularly under a Mediterranean-type climate. Further studies accounting with vitality proxies, grape quality and inter-annual variability are needed to better support this hypothesis. WUE adjustments to drought were common among the varieties, but much more pronounced in certain varieties (great stomatal regulation highlighted by high phloem $\delta^{13}C$ in non-irrigated rainfed conditions and increased ^{13}C enrichment when comparing full irrigation to non-irrigated conditions). These results underpin the importance of isotopic measures, and phloem $\delta^{13}C$ as a complementary promising tool to assess short and long-term (seasonal and annual) responses to drought in grapevines.

With these results, we aim to contribute to pinpoint drought-adapted grapevine varieties, by providing a potentially useful list of varieties that, by being able to phsyiologically adjust and increase their WUE under drought conditions, might be consider more acquitted to deal with future drought and sustainable water-management scenarios.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data used in this research is shared as suplementary material (Table A2). Raw data will be made available upon request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agwat.2023.108396.

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