Research Article

Received: 9 June 2022 Revised: 23 September 2022 Accepted article published: 5 November 2022 Published online in Wiley Online Library: 21 November 2022

(wileyonlinelibrary.com) DOI 10.1002/jsfa.12316

Insights into the stable isotope ratio variability of hybrid grape varieties: a preliminary study

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Abstract

BACKGROUND: Official stable isotope databases, based on the analysis of $(D/H)_{I\ ethanol}$, $(D/H)_{II\ ethanol}$, $\delta^{13}C_{ethanol}$ and $\delta^{18}O_{water}$ of wine, are an indispensable tool for establishing the limits beyond which the mislabeling or the addition of sugar and/or water in wine production can be detected. The present study investigates, for the first time, whether the use of hybrid varieties instead of European *Vitis vinifera* for wine production can have an impact on the stable isotope ratios.

RESULTS: The analyses were performed by isotope ratio mass spectrometry and site-specific natural isotope fractionation by nuclear magnetic resonance, in accordance with the official methods of the International Organization of Grapes and Wine. The comparison shows the tendency of some stable isotope ratios of hybrid varieties, in particular $(D/H)_I$, to deviate from the regional averages of the *V. vinifera* samples. Notably, Baron, Monarch and Regent showed significantly different values at one of the two sampling sites. Particularly high $\delta^{13}C$ values characterize Helios compared to other hybrid varieties.

CONCLUSION: For the first time, and from an isotopic point of view, the present study investigates the wine obtained from hybrid varieties, showing that further attention should be paid to their interpretation, on the basis of the database established according to the European Regulation 2018/273.

Supporting information may be found in the online version of this article.

Keywords: stable isotope; SNIF-NMR; IRMS; hybrid; grape

INTRODUCTION

European *Vitis vinifera* varieties have always been known and widely used for their excellent grape quality. A threat to this cultivation is represented by a wide range of diseases and pests, such as powdery mildew (*Erysiphe necator*), downy mildew (*Plasmopara viticola*), botrytis (*Botrytis cinera*) and Pierce's disease (*Xyllela fastidiosa*), which require specific plant protection measures[1] A survey on the use of plant protection products in the European Union[2] showed that, although vines cover only approximately 3% of the agricultural land, they account for approximately 65% of the total European Union market (68 000 tonnes year^{-1}) in plant protection products. A large part of the applied quantities goes back to the control of powdery mildew. In this respect, the influence of climate change will have a further negative impact on possible fungicide reductions and, according to Salinari *et al.*,[3] the change in temperature and precipitation conditions promote the growth of *P. viticola*. In addition, the development of resistance to fungicides is a recurring issue.[4] On the other hand, the wine industry faces the consumer's increasing demand for a sustainable and environmental-friendly production.[5] This request has been shared and boosted by the European Union within the European Green Deal in the Farm to Fork strategy, which aims to reduce pesticide utilization in farming systems by 50%.[6] Among the agronomic approaches so far proposed, the use of mould-resistant hybrid varieties (based on crossings of *V. vinifera* with other *Vitis* species) with a high tolerance to vine pathogens attacks is gaining the vine growers attention and their production is continuously increasing.[7]

In this general framework, the analysis of stable isotope ratios is the reference method for revealing counterfeiting in the wine sector (such as watering down or addition of exogenous sugars).[8] Nevertheless, the way in which the isotope ratios of wine can be influenced by the increasing use of these hybrid varieties has not yet been explored. Stable isotope ratio analysis is based on the measurement of the ratio between the heavier and the lighter isotope of a specific element (e.g. C, H, or O). These elements become enriched or depleted in their heavier/lighter isotope

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through a variety of kinetic and thermodynamic isotope effects, the so-called 'non-statistical intermolecular distribution of stable isotopes'.[9] These phenomena are regulated by several well-known processes, classified according to their biotic (e.g. Calvin pathway of C_3 plants *versus* the Hatch-Slack pathway of C_4 plants[10]) and abiotic (e.g. evapotranspiration from grapes and leaves[11]) causes. Therefore, the measurement of isotope ratios can be used to discriminate between samples otherwise sharing identical chemical compositions.[12]

Official methods applied in the wine chain are based on the analysis of ethanol in wine products after distillation, aiming to identify illegal additions of exogenous sugars (such as beet or cane). These analyses consist in the determination of the site-specific D/H ratio (OIV-MA-AS311-05 R2011) using Site-specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) and of the $^{13}C/^{12}C$ stable isotope ratio (OIV-MA-AS312-06 R2009) using isotope ratio mass spectrometry (IRMS).[13] In the official method OIV MA-AS2-12 R2009, the ratio of the stable isotopes of water oxygen ($^{18}O/^{16}O$, expressed as $\delta^{18}O$) is used to identify fraudulent additions of water to wine and to verify the geographical origin and the year of harvest declared for the product. This can be achieved by building annual official reference databases, in which the isotopic ranges of variability of authentic wine are reported.[8]

The response of vines to water stress is a cultivar-dependent feature,[14] which can also alter the isotopic ratios.[15] Although the metabolic spectrum of hybrid varieties overlaps the *V. vinifera* one,[16] the wines from resistant hybrids show peculiar characteristics depending on the variety linked to the genotype[17] and to its interaction with the environment.[18,19] The combination of these synergetic features requires further investigation, also considering recent approval by the European Parliament to the use of grape varieties resistant to diseases in products having a designation of origin (European Regulation 2021/2117).

The present study aims to show and discuss, for the first time, the stable isotope ratios of H, C and O of wines produced using hybrid grape varieties and, subsequently, to determine whether the use of disease-resistant varieties instead of European *V. vinifera* may affect the stable isotopic ratio variability of wine. In this case, the isotopic database, established according to European Regulation 2018/273, should also consider the possible use of hybrid varieties. Wine samples produced by seven white hybrid varieties (Aromera, Bronner, Helios, Johanniter, Muscaris, Solaris, Souvignier Gris) and seven red hybrid varieties (Baron, Cabernet Cortis, Cabernet Cantor, Cabernet Carbon, Monarch, Prior, Regent) grown in two experimental plots sited in the northern Italian region of Trentino and sampled in three different wine years (2017, 2018 and 2019) were considered. Furthermore, as a comparison, samples of wine from *V. vinifera* variety from the same region (Trentino) and sampled in the same 3 years were analyzed.

MATERIALS AND METHODS

Samples

Sixty-nine samples from 14 mold-resistant hybrid grape varieties, bred and selected in the State Institute for Viticulture Freiburg (Germany) and the Geilweilerhof Institute for Plant Breeding (Germany), were grown in two experimental vineyards (Navicello and Telve) sited in Trentino (Italy) (for specifications, Supporting information, Fig. S1). The experimental vineyards were geographically differentiated, having specific pedoclimatic features and an agronomic management performed according to the production goals. The Telve plot faces south and has an average altitude of 415 m a.s.l. and an extension of approximately 0.4 ha with a

Table 1. A selection of 14 hybrid grape varieties and their day and year of harvest

	Navicello	2017	2018	2019	Telve	2017	2018	2019
WHITE	Aromera	12 September	11 September	16 September	–	–	–	–
	Bronner	30 August	29 August	16 September	Bronner	10 september	5 September	25 September
	Helios	2 September	31 August	–	Helios	–	5 September	18 September
	Johanniter	2 September	31 August	9 September	Johanniter	15 September	13 September	23 September
	Muscaris	8 September	6 September	9 September	Muscaris	12 September	11 September	12 September
	Solaris	2 September	27 August	2 September	Solaris	29 August	27 August	5 September
	Souvignier Gris	26 September	26 September	24 September	Souvignier Gris	28 September	26 September	30 September
RED	Baron	8 september	6 September	12 September	Baron	10 September	5 September	25 September
	Cabernet Cantor	10 September	13 September	9 September	Cabernet Cantor	–	13 September	16 September
	Cabernet Carbon	–	19 September	16 September	Cabernet Carbon	28 September	26 September	30 September
	Cabernet Cortis	–	10 September	12 September	Cabernet Cortis	18 September	17 September	16 September
	Monarch	–	19 September	23 September	Monarch	–	20 September	30 September
	Prior	–	19 September	16 September	Prior	22 September	20 September	25 September
	Regent	8 September	6 September	–	Regent	–	5 September	25 September

Table 2. Mean isotopic parameters determined in white and red wines in three vintages (2017–2018–2019) from the Navicello plot

Navicello white	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V-PDB	$\delta^{18}O$ ‰ *versus* V-SMOW
Aromera (*n* = 3)	103.1 a	129.4 a	−28.8 ab	3.1 a
Bronner (*n* = 3)	103.5 a	126.0 a	−27.8 bc	2.9 a
Helios (*n* = 2)	104.0 a	126.6 a	−27.2 c	3.9 a
Johanniter (*n* = 2)	104.0 a	125.6 a	−29.3 a	3.7 a
Muscaris (*n* = 3)	102.6 a	127.4 a	−28.3 abc	2.4 a
Solaris (*n* = 3)	102.8 a	128.9 a	−28.6 abc	4.4 a
Souvignier Gris (*n* = 3)	102.8 a	128.7 a	−28.4 abc	2.7 a
Navicello red	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V-PDB	$\delta^{18}O$ ‰ *versus* V-SMOW
Baron (*n* = 3)	104.4 a	131.1 a	−27.3 a	4.2 a
Cabernet Cantor (*n* = 4)	103.4 a	131.1 a	−27.4 a	3.7 a
Cabernet Carbon (*n* = 2)	104.0 a	128.9 a	−27.3 a	3.4 a
Cabernet Cortis (*n* = 2)	102.5 a	130.3 a	−27.4 a	3.3 a
Monarch (*n* = 2)	104.3 a	129.9 a	−28.0 a	4.5 a
Prior (*n* = 1)	101.7 a	130.8 a	−28.3 a	3.9 a
Regent (*n* = 2)	105.9 a	131.1 a	−28.1 a	4.7 a

Note: Different lowercase letters in the same column indicate significant differences by ANOVA and Tukey's test (95%).

slightly steep trend. The vines are trained in Guyot. The Navicello plot is sited on the bottom of the Adige valley and has an average altitude of 170 m a.s.l. and an extension of approximately 1.2 ha with a flat layout. The vines are trained on the simple pergola system.[20]

Twenty-eight samples from different *V. vinifera* grape varieties (see Supporting information, Table S1) were grown in different vineyards in Trentino (Italy) and sampled in three different harvest years: 2017, 2018 and 2019.

Wines were produced according to two different experimental protocols, depending on the color of the skin and the wine style. Briefly, white wines were destemmed, pressed and settled for 24 h. The alcoholic fermentation was conducted with a dry active yeast inoculated after must racking. At the end of the alcoholic fermentation, sulfur dioxide was added to the wine, which was then stored at 4 °C until filtration and bottling. On the other hand, red wines were destemmed with the addition of sulfur dioxide and were therefore inoculated with dried active yeast. Skin contact maceration was performed punching down the skins twice a day for 7 days. Marcs were pressed and the resulting wine was inoculated with lactic acid bacteria for malolactic fermentation. After settling, wines were filtered and bottled. Further information about the vinification protocols has been reported previously.[19,21] All wines were stored at 14 °C until analysis.

The varieties, the sampling sites (trace) and the harvest dates in the three considered years are shown in Table 1.

Stable isotope analysis

Distillation of wine and SNIF-NMR analysis

The samples were first distilled using a Cadiot spinning band column according to official OIV methods (MA-AS311-05 MA-AS-312-06). This system offers a high distillation efficiency and avoids isotope fractionation during the process by preventing evaporation of water after the complete distillation of the alcohol.

Table 3. Mean isotopic parameters determined in white and red wines in three vintages (2017–2018–2019) from the Telve plot

Telve White	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V-PDB	$\delta^{18}O$ ‰ *versus* V-SMOW
Bronner (*n* = 3)	101.1 a	125.2 a	−28.2 ab	1.4 a
Helios (*n* = 2)	102.4 a	127.4 a	−26.7 c	0.9 a
Johanniter (*n* = 3)	101.7 a	124.2 a	−28.5 ab	1.0 a
Muscaris (*n* = 3)	100.6 a	127.4 a	−28.5 ab	1.4 a
Solaris (*n* = 3)	100.7 a	127.2 a	−28.9 a	2.5 a
Souvignier Gris (*n* = 3)	101.5 a	127.9 a	−27.9 b	1.0 a
Telve Red	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V-PDB	$\delta^{18}O$ ‰ *versus* V-SMOW
Baron (*n* = 3)	102.5 a	128.9 a	−28.4 a	1.5 a
Cabernet Cantor (*n* = 2)	101.0 a	129.8 a	−27.3 a	1.8 a
Cabernet Carbon (*n* = 3)	102.0 a	130.1 a	−27.7 a	1.1 a
Cabernet Cortis (*n* = 2)	101.1 a	129.0 a	−27.5 a	1.1 a
Monarch (*n* = 2)	102.8 a	125.3 a	−28.2 a	1.3 a
Prior (*n* = 3)	102.3 a	129.6 a	−27.7 a	1.5 a
Regent (*n* = 2)	102.6 a	127.6 a	−28.3 a	1.0 a

Note: Different lowercase letters in the same column indicate significant differences by ANOVA and Tukey's test (95%).

Table 4. Mean isotopic parameters determined in white wine in three vintages (2017–2018–2019) and two plots (Navicello + Telve)

White wines	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V-PDB	$\delta^{18}O$ ‰ *versus* V-SMOW
Aromera (*n* = 3)	103.1 a	129.4 a	−28.8 b	3.1 a
Bronner (*n* = 6)	102.6 a	125.6 bc	−28.0 b	2.1 a
Helios (*n* = 4)	103.3 a	127.0 abc	−26.9 a	2.4 a
Johanniter (*n* = 5)	102.6 a	124.8 b	−28.8 b	2.1 a
Muscaris (*n* = 6)	101.6 a	127.4 abc	−28.4 b	1.9 a
Solaris (*n* = 6)	101.7 a	128.1 ac	−28.8 b	3.5 a
Souvignier Gris (*n* = 6)	102.2 a	128.3 a	−28.2 b	1.9 a

Note: Different lowercase letters in the same column indicate significant differences by ANOVA and Tukey's test (95%).

The samples of ethanol were analyzed using SNIF-NMR (FT-NMR AVANCE III 400; Bruker BioSpin GmbH, Karlsruhe, Germany). The D/H values were measured site-specifically in the methyl and methylene sites of ethanol [$(D/H)_I$ and $(D/H)_{II}$]. These results are expressed in parts per million (ppm).

The isotopic values were calculated against certified tetramethylurea 99% STA-003 m with a value of 153.70 ppm (JRC Institute for Reference Materials and Measurements, Geel, Belgium) for $(D/H)_I$ and $(D/H)_{II}$. Analytical uncertainties of measurement (calculated as 2 standard deviations of reproducibility of the measurements alone) were lower than 0.8 ppm for $(D/H)_I$ and 1.2 ppm for $(D/H)_{II}$ in ethanol.

IRMS analysis

The $\delta^{13}C$ measurement of wine ethanol was performed using an IRMS (SIRA II; VG ISOGAS; FISIONS, Rodano, Milano, Italy) interfaced with an Elemental Analyzer (Flash 1112; Carlo Erba, Milano, Italy) in accordance with the OIV-MA-AS312-06 method.

The $\delta^{18}O$ measurement of wine water was performed according to the OIV-MA-AS2-12 method. An IRMS (SIRA II; VG Fisons, Middlewich, UK), connected to the water/CO_2 equilibration system Isoprep 1 (VG Fisons) was used for the analysis.

According to the IUPAC protocol,[22] the $^{13}C/^{12}C$ and $^{18}O/^{16}O$ values are expressed in the delta scale (δ‰), against the international standards V-PDB (Vienna-Pee Dee Belemnite) for carbon and VSMOW/SLAP (Vienna-Standard Mean Ocean Water/Standard Light Antarctic Precipitation) for oxygen according to:

$$\delta_{ref}\left({}^{i}E/{}^{j}E,\ sample\right)=\left[\frac{R\left({}^{i}E/{}^{j}E,\ sample\right)}{R\left({}^{i}E/{}^{j}E,\ ref\right)}\right]-1 \quad (1)$$

where *ref* is the international measurement standard, *sample* is the analyzed sample and ${}^{i}E/{}^{j}E$ is the isotope ratio between heavier and lighter isotopes. The delta values were multiplied by 1000 and expressed in units 'per mil' (‰).

The isotopic values were calculated against in-house working standards (ethanol and water), calibrated against international reference materials: ethanol BCR 656 (Institute for Reference Materials and Measurements, Geel, Belgium) with a value of −26.91 ± 0.07 ‰ and fuel oil NBS-22 (International Atomic Energy Agency, Vienna, Austria) with a value of −30.03 ± 0.04‰ for $\delta^{13}C$, as well as V-SMOW2 with a value of 0.0 ± 0.02‰ and SLAP2 with a value of −55.50 ± 0.02‰ (International Atomic Energy Agency) for $\delta^{18}O$. Analytical uncertainties of measurement (calculated as 2 standard deviation of reproducibility of the measurements alone) were lower than 0.30 ‰ for $\delta^{18}O$ in water, 0.30 ‰ for $\delta^{13}C$ in ethanol.

Statistical analysis

The data were statistically evaluated using XLSTAT (Addinsoft, Paris, France). Statistical differences were revealed through the representation of data with box and whisker plots (graphically displaying the median, minimum and maximum of the dataset variables) or through regression analysis, considering a confidence level of 95%. One-way analysis of vatiance (ANOVA) was performed to determine the significant spatial difference of variables. Tukey's honestly significant difference test for unequal sample sizes was considered to evaluate significant differences according to geographical origin. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Variation in stable isotope ratios by variety

To evaluate the potential cultivar effect, the $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ of ethanol and $\delta^{18}O$ of wine water resulting from the analysis of the hybrid varieties were compared. The use of different yeast strains during vinification has no significant effect on the D/H and $\delta^{13}C$

Table 5. Mean isotopic parameters determined in red wine in three vintages (2017–2018—2019) and two plots (Navicello + Telve)

Red wines	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V—PDB	$\delta^{18}O$ ‰ *versus* V—SMOW
Baron (*n* = 6)	103.4 a	130.0 a	−27.9 a	2.9 a
Cabernet Cantor (*n* = 6)	102.6 a	130.7 a	−27.4 a	3.1 a
Cabernet Carbon (*n* = 5)	102.8 a	129.6 a	−27.5 a	2.0 a
Cabernet Cortis (*n* = 4)	101.8 a	129.6 a	−27.5 a	2.2 a
Monarch (*n* = 4)	103.7 a	127.6 a	−28.1 a	2.9 a
Prior (*n* = 4)	102.2 a	129.9 a	−27.9 a	2.1 a
Regent (*n* = 4)	104.3 a	129.3 a	−28.2 a	2.8 a

Note: Different lowercase letters in the same column indicate significant differences by ANOVA and Tukey's test (95%).

Table 6. Mean isotopic values of white wines from two considered plots (Navicello and Telve) in 2017, 2018 and 2019

2017 White	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Aromera (*n* = 1)	102.0 a	128.0 a	−28.6 a	4.5 a
Bronner (*n* = 2)	101.9 a	126.0 a	−28.5 a	3.5 a
Helios (*n* = 1)	102.8 a	128.3 a	−27.4 a	4.1 a
Johanniter (*n* = 2)	101.7 a	124.9 a	−28.9 a	2.9 a
Muscaris (*n* = 2)	101.2 a	127.5 a	−28.6 a	3.9 a
Solaris (*n* = 2)	101.4 a	129.3 a	−29.2 a	4.6 a
Souvignier Gris (*n* = 2)	101.4 a	127.3 a	−28.1 a	0.9 a
2018 White	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Aromera (*n* = 1)	105.3 a	130.1 a	−29.1 ab	2.5 a
Bronner (*n* = 2)	103.3 a	124.4 a	−27.6 ab	1.9 a
Helios (*n* = 2)	104.1 a	125.8 a	−26.7 a	2.1 a
Johanniter (*n* = 2)	104.0 a	124.9 a	−29.1 b	1.8 a
Muscaris (*n* = 2)	102.6 a	126.8 a	−28.1 ab	0.9 a
Solaris (*n* = 2)	102.6 a	126.0 a	−28.5 ab	3.2 a
Souvignier Gris (*n* = 2)	103.3 a	128.5 a	−28.0 ab	3.1 a
2019 White	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Aromera (*n* = 1)	101.9 a	130.0 a	−28.6 a	2.2 a
Bronner (*n* = 2)	101.8 a	126.5 a	−28.0 a	1.1 a
Helios (*n* = 1)	101.7 a	128.0 a	−27.0 a	1.2 a
Johanniter (*n* = 1)	101.9 a	124.5 a	−28.2 a	1.2 a
Muscaris (*n* = 2)	101.1 a	128.0 a	−28.5 a	0.9 a
Solaris (*n* = 2)	101.2 a	128.9 a	−28.7 a	2.6 a
Souvignier Gris (*n* = 2)	101.8 a	129.1 a	−28.5 a	1.6 a

Note: Different lowercase letters in the same column indicate significant differences by ANOVA and Tukey's test (95%).

Table 7. Mean isotopic values of red wines from two considered plots (Navicello and Telve) in 2017, 2018 and 2019

2017 Red	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Baron (*n* = 2)	103.3 a	131.7 a	−27.8 a	5.0 a
Cabernet Cantor (*n* = 1)	104.2 a	131.9 a	−27.5 a	6.6 a
Cabernet Carbon (*n* = 2)	104.2 a	130.5 a	−27.3 a	1.1 a
Cabernet Cortis (*n* = 1)	100.7 a	127.6 a	−27.9 a	0.4 a
Prior (*n* = 1)	101.8 a	128.4 a	−28.2 a	1.4 a
Regent (*n* = 1)	105.0 a	130.8 a	−28.5 a	5.4 a
2018 Red	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Baron (*n* = 2)	103.9 a	130.2 a	−27.7 a	1.3 a
Cabernet Cantor (*n* = 2)	104.0 a	130.8 a	−27.3 a	2.6 a
Cabernet Carbon (*n* = 2)	104.0 a	129.9 a	−27.4 a	2.5 a
Cabernet Cortis (*n* = 1)	103.3 a	130.3 a	−27.2 a	3.1 a
Monarch (*n* = 2)	104.6 a	126.4 a	−28.0 a	3.3 a
Prior (*n* = 1)	103.2 a	130.8 a	−27.7 a	1.7 a
Regent (*n* = 2)	104.9 a	130.5 a	−27.8 a	2.3 a
2019 Red	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Baron (*n* = 2)	103.1 a	128.2 a	−28.1 a	2.3 a
Cabernet Cantor (*n* = 2)	100.6 b	130.1 a	−27.4 a	2.7 a
Cabernet Carbon (*n* = 2)	102.3 ab	129.2 a	−27.8 a	2.1 a
Cabernet Cortis (*n* = 1)	101.6 ab	130.3 a	−27.4 a	3.4 a
Monarch (*n* = 2)	102.7 ab	128.8 a	−28.2 a	2.5 a
Prior (*n* = 2)	101.8 ab	130.3 a	−27.8 a	2.7 a
Regent (*n* = 1)	102.3 ab	125.4 a	−28.6 a	1.3 a

Note: Different lowercase letters in the same column indicate significant differences by ANOVA and Tukey's test (95%).

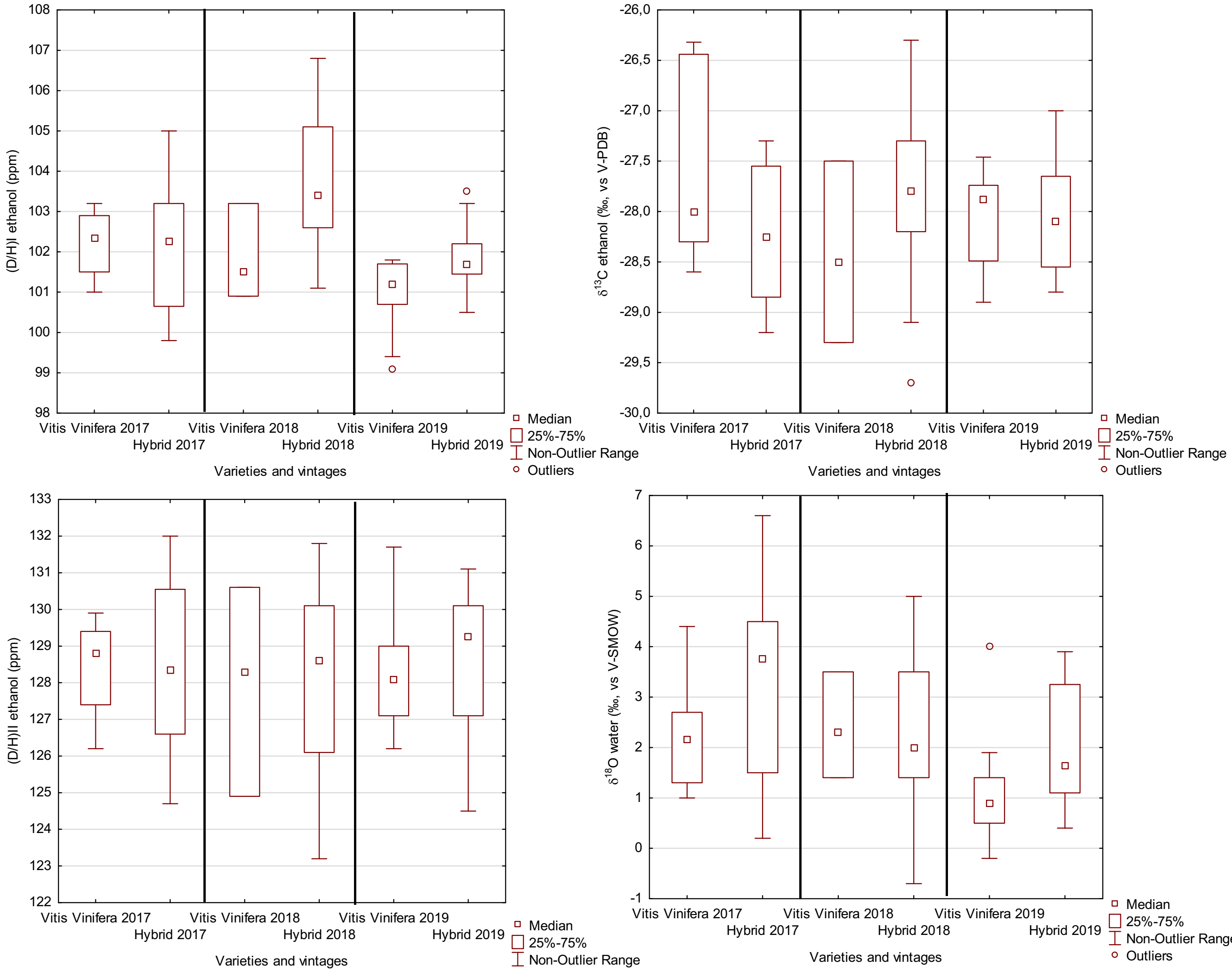

Figure 1. Variation of $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ and $\delta^{18}O$ content in hybrid and *Vitis vinifera* varieties in three different years (2017, 2018 and 2019).

values, according to Fauhl and Wittkowski,[23] and is therefore disregarded as a factor of isotopic variability in the subsequent evaluation of the results.

Wine samples were tested for significant differences using ANOVA and Tukey's test (95%) of the mean values, considering white and red wine separately in each of the vintages (2017, 2018 and 2019) within the respective plot (Navicello and Telve) (Tables 2 and 3) and white and red wines separately over the 3 years and the two plots (Tables 4 and 5).

The results of the Navicello plot (Table 2) show that there are generally no significant differences within the hybrid grape varieties, either for white or for red wines. The isotope parameter $\delta^{13}C$ is the only one that shows a statistically significant difference between the hybrid variety Helios and the other white wine varieties. This could be explained by the effect of post-veraison water stress described by Poni *et al.*,[15] which results in relatively high $\delta^{13}C$ values. The results obtained could indicate a greater sensibility of the Helios variety to water stress, although only more in-depth studies can confirm this hypothesis.

No significant differences were found among the red wines from the Telve plot (Table 3). Once again, for white wines, the $\delta^{13}C$ made it possible to distinguish the hybrid Helios from the other white wine varieties.

In Table 4, an evaluation of significant differences among mean values of white wines regardless of their origin (Navicello and Telve) is reported. Significant differences in the $(D/H)_{II}$ and $\delta^{13}C$ values of some grape varieties are highlighted. The former parameter made it possible to discriminate Aromera and Souvignier Gris from Johanniter, whereas the latter revealed a significant difference between Helios and the other considered varieties.

None of the wines in Table 5, comparing the mean values of all red varieties regardless of their origin, differed significantly from each other.

Variation in isotope ratios by year of harvest

To determine whether there is a significant difference among the grape varieties for each vintage, the samples were tested using ANOVA and Tukey's test (95%). As shown in the Supporting information (Fig. S2), the climatic conditions (average annual temperature), monitored at the weather station at San Michele all'Adige (Trento, Italy) were not significantly different between the 2 years (2018 and 2019) and in line with the historical trend. A distinction

Table 8. Deviations to the isotopic parameters from the respective reference variety (Chardonnay: White Wines; Marzemino: Red Wines) in the Navicello plot in two vintages (2018–2019)

Origin, year of harvest and color of grape		Variety	$(D/H)_I$ ppm	$(D/H)_{II}$ ppm	$\delta^{13}C$ ‰ *versus* V–PDB	$\delta^{18}O$ ‰ *versus* V–SMOW
Navicello 2018	White	RV*: Chardonnay	101.5	124.9	−29.3	1.4
		Deviations to the RV*:				
		Solaris	**2.3**	**1.2**	**1.1**	**3.2**
		Bronner	**3.4**	0.1	**2**	**2.1**
		Helios	**3.6**	0	**2.3**	**2.3**
		Johanniter	**3.8**	**1.6**	−0.4	**2**
		Aromera	**3.8**	**5.2**	0.2	**1.1**
		Muscaris	**2.5**	**1.9**	**1.6**	0
		Souvignier Gris	**2.7**	**3**	**1.3**	**2.8**
	Red	RV*: Marzemino	103.2	130.6	−27.5	3.5
		Deviations to the RV*:				
		Baron	**2**	0.9	**0.6**	−0.2
		Cabernet Cantor	**1**	**1.2**	**0.4**	0.2
		Cabernet Carbon	**1.9**	**−2**	**0.7**	−0.1
		Cabernet Cortis	0.1	−0.3	0.3	**−0.4**
		Monarch	**2.6**	**−2**	−0.3	**1.5**
		Regent	**3.6**	0.7	−0.1	**0.4**
Navicello 2019	White	RV*: Chardonnay	100.9	127.3	−28.6	1.2
		Deviations to the RV*:				
		Aromera	**1**	**2.7**	0	**1**
		Bronner	**1.3**	0.7	**0.7**	**0.5**
		Muscaris	0.5	−0.6	0.3	0.1
		Solaris	0.6	**3**	0.1	**2.3**
		Souvignier Gris	**1.1**	**2.4**	−0.2	**1.1**
	Red	RV*: Marzemino	101.8	131	−28.4	1.4
		Deviations to the RV*:				
		Baron	**1.7**	−1.1	**0.8**	**2.4**
		Cabernet Cantor	**−1.1**	−0.8	**0.7**	**1.8**
		Cabernet Carbon	**1**	**−1.8**	**0.6**	**1.9**
		Cabernet Cortis	−0.2	−0.7	**0.8**	**2**
		Monarch	**1.4**	0.1	0.3	**2.5**
		Prior	−0.1	−0.2	0.1	**2.5**

between white (Table 6) and red vine varieties (Table 7), regardless of the origin of the sample, was considered.

As shown in Table 6, the $\delta^{13}C$ value represented the only exception for significance in the 2018 among the white wines and revealed a significant difference between the Helios and Johanniter hybrid grape varieties. As for 2017 and 2019, the Helios variety showed particularly high values of $\delta^{13}C$ (−27.4‰ and −27.0‰, respectively), although not significant, compared to all the other varieties considered.

Focusing on the statistical analysis of the red wines in all vintages (Table 7), no significant differences were found among the cultivars, except in 2019 $(D/H)_I$ values. The hybrid Cabernet Cantor shows the lower value of $(D/H)_I$ (100.6 ppm), which is significantly different from the Baron hybrid variety (103.1 ppm).

Deviation of isotope ratios from hybrids to *V. vinifera*

Figure 1 shows the distribution in box and whisker plots of the $(D/H)_I$, $(D/H)_{II}$, $\delta^{13}C$ and $\delta^{18}O$ values of the samples of *V. vinifera* and hybrid varieties in the three considered vintages (2017, 2018 and 2019, respectively). ANOVA and Tukey's test do not show statistically significant differences between the two groups considered in the different years ($P > 0.01$).

By observing the different distributions, it is possible to observe how some specific varieties, even considering the analytical uncertainty associated with the different parameters, had values falling outside of the range of variability established on the basis of *V. vinifera* samples (mean ± 2 SD) (see Supporting information, Table S1). The values of these samples also laid outside the range of variability reported by Camin *et al.* for the Trentino region,[24] which can be considered as a reference regardless of the harvest year. The validity of the values for a certain region despite of the harvest year has been reported by Ogrinc *et al.*[25] as for the $(D/H)_I$ and $\delta^{13}C$ values.

Regardless of the harvest year, the hybrid samples of the Baron, Regent and Monarch varieties grown in the Navicello plot stood out with very high values of $(D/H)_I$. In 2018, characterized by an upper limit for *V. vinifera* varieties of 104.3 ppm, we found values for the three hybrid varieties equal to 105.2 ppm for Baron, 105.8 ppm for Monarch and 106.8 ppm for Regent. The same hybrid varieties grown in the Telve parcel did not show this behavior, indicating that these deviations could be caused by a blend of conditions (varieties and environmental conditions).

Considering the analytical uncertainty of 1.2 ppm, none of the hybrid samples showed a value of $(D/H)_{II}$ outside the variability range of the *V. vinifera* samples for the respective three considered years (see Supporting information, Table S1).

Although remaining within the regional variability for the individual years considered, the hybrid varieties Helios and Cabernet Cortis had values of δ^{13}C laying at the upper limits of the 2018 (−26.3‰) and 2019 (−26.9‰) vintages of the *V. vinifera* samples.

In the 2017 vintage, it was possible to calculate a limit value for the parameter δ^{18}O equal to +4.5 ‰. Various hybrid varieties coming from the Navicello plot had values exceeding this limit and, in particular, the hybrid Carbernet Cantor reported a value of +6.6 ‰.

This behavior was not repeated in the 2018 and 2019 vintages, when almost all the hybrid varieties fell within the calculated variability range. An exception was represented by the Monarch hybrid, which, in 2018, had a value of +5.0 ‰, above the limit of +4.8 ‰ (uncertainty included), and, in 2019, reached the limit (showing a value of +3.9 ‰ and being the limit set at +4 ‰).

The results appeared to indicate a possible difference between *Vitis Vinifera* and some hybrid varieties, but further analyses should be carried out to verify this assumption. Indeed, even phenomena of biotic and abiotic fractionation can modify the isotopic composition of grapes.

By considering only the white and red hybrid wine samples of Navicello plot (2018 and 2019), it is possible to compare their isotopic data $(D/H)_I$, $(D/H)_{II}$, δ^{13}C and δ^{18}O with the *V. vinifera* reference varieties Chardonnay and Marzemino (marked withan asterisk) grown in the same plot and in the same conditions (Table 8). All values with a deviation above the analytical uncertainty of the respective parameter (see Stable isotope analysis in the Materials and methods) are indicated in bold. Individual values are reported in the Supporting information (Table S1).

The $(D/H)_I$ values of the Navicello hybrid samples ranged between 103.3 and 106.8 ppm (Regent sample) and showed higher values than the reference varieties, particularly in 2018.

In 2018, the Aromera hybrid showed a larger deviation (+5.2 ppm) of $(D/H)_{II}$ with respect to the reference variety, with the $(D/H)_{II}$ values of hybrid Navicello samples ranging between 124.4 and 131.5 ppm.

The δ^{13}C values of hybrid Navicello samples ranged between −29.7 ‰ and −26.8 ‰. Once again, higher deviations of the hybrids compared to the reference variety were mostly recorded in 2018, with deviations much higher than the analytical uncertainty (0.3 ‰) especially for Helios (+2.3 ‰ *versus* Chardonnay) and for Bronner (+2 ‰ *versus* Chardonnay).

The δ^{18}O values, ranging between +1.4 ‰ and +5 ‰ for hybrid Navicello samples, showed high deviations in both years, especially in 2018. The maximum difference is reported by the Solaris variety (+3.2 ‰ respect to Chardonnay). The Monarch and Solaris varieties showed particularly high values of 5.0 ‰ and 4.6 ‰ in 2018 (see Supporting information, Table S1). They are normally harvested earlier than the other varieties when the climate is warmer and drier after veraison. This contributed to particularly high values, which are typical of southern Italy, but not common for the wine-growing areas of Trentino.

CONCLUSIONS

The results of the present study showed that the stable isotope ratios $(D/H)_I$, $(D/H)_{II}$, δ^{13}C and δ^{18}O of some hybrid grape varieties differed from each other within the same year and/or regardless of the respective year and origin. The differences mainly concerned D/H_{II} and δ^{13}C. Red grape varieties such as Monarch, Baron and Regent showed ethanol $(D/H)_I$ values out of the range of variability reported in the literature for Trentino, and out of the extremes calculated on the basis of authentic samples of *V. vinifera* for the same region and year.

Based on the present study, it was not possible to establish whether these results were related to the grape variety or resulted from abiotic/biotic isotope fractionation effects. In any case, these preliminary results show that further attention must be paid to the interpretation of stable isotope data measured on hybrid wines compared to the database established according to the European Regulation 2018/273. This initial study should be extended over time to provide more meaningful results by considering a larger number of samples.

ACKNOWLEDGEMENTS

This work was supported by the VEViR ('*Valorizzazione enologica di vitigni resistenti*') project, through action n. 16 of the Rural Development Programme 2014-2020 of the Trentino Province. The project aimed to identify varieties most suitable for cultivation in certain areas of the Trentino Province to outline the technical protocols for cultivation and winemaking and to assess the relevant economic sustainability.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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