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Phenolic potential of new red hybrid grape varieties to produce quality wines and identification by the malvin

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Abstract. The hybrids are new varieties that are resistant to the cryptogamic diseases of vines, and they may be a good solution to the reduction of pesticide use. However, these new varieties have appeared recently and only few studies have been conducted to check the quality of the wine that they produce. In this study, wines originated from hybrids were chemically characterized (polyphenols, tannins, anthocyanins, and color analysis). Results show that their oenological parameters were close to those of the *Vitis vinifera* wines. The wines made from Vinifera (100%), with Vidoc, and all the resistant Bouquets studied are malvidin-3 glucoside dominant, and all the others hybrids varieties are cyanidin dominant. Furthermore, the hybrid wines had a higher concentration in condensed tannins and a lower one in molecular tannins compared with the *Vitis vinifera* wines, except for the Vidoc wines. As far as the anthocyanin content is concerned, the samples contained anthocyanin diglucosides, with the malvidin-3,5-diglucoside (malvin) the most abundant one. Also, their concentration in molecular anthocyanins were higher than the concentration in normal wines. The Vidoc wines do not contain any malvin. For the other wines, malvin co-eluted with cyanidin-3-*O*-glucoside and in this case, the cyanidin's concentration was impressively higher than the Vidoc wines. The current OIV method gives different results for malvidol diglucoside compared to the use of an HPLC chromatography method (HPLC-DAD-QQQ). The current OIV method does not appear reliable in comparison with the HPLC method for detecting malvin. Finally, the hybrid wines had a darker blue/purplish color than the *Vitis vinifera* wines.

1 Introduction

Hybrid grape varieties are varieties that come from the cross between Asian/American vines (Vitis labrusca, Vitis amurensis, Vitis rupestris) and European vines (Vitis vinifera). Historically, hybrid varieties appeared in the 19th century to fight against phylloxera, but ultimately these grape varieties were not retained for qualitative reasons [1, 2]. However, in recent years, the use of hybrids from new crosses appears as a possible solution to problems in the wine sector such as reducing the use of pesticides or climate change. In order to face the weaknesses of some traditional cultivars, current trends in viticulture and oenology research concern the exploration of new grape varieties resistant to cryptogamic diseases.

Each grape variety presents an own phenolic fingerprint, as well as a particular antioxidant capacity and aroma precursors that could also significantly differ from one to another [3, 4]. Thus, research on the phenolic and volatile profile, as well as the consumers' acceptance, of wines produced from hybrid grape varieties may represent a significant step forward in supporting their promotion in winemaking.

According to the literature, apart from the genetic differences between wild and traditional *Vitis*, wines made from hybrid grape varieties have a different chemical composition from that of wines made from classic grape varieties. One of the interesting characteristics of this type of wine is the detection of anthocyanin diglucosides, with malvidin-3,5-diglucoside being the most abundant [5, 6]. Our results of 2019 showed that the level of malvidin-3-glucoside appears to be linked to the % of vinifera genome in the varieties studied. Varieties containing less than 81% of Vitis vinifera genome had anthocyanins mainly dominated by cyanidin [6]. The molecule of malvidin-3,5-diglucoside has been indicated as a molecule that can cause health problems if its concentration is high enough [7]. The International Organization of Vine and Wine (OIV) has set a regulatory limit of 15 mg/L of malvidin-3,5-diglucoside in commercial wines.

This study therefore aims to expand knowledge on the wines resulting from hybrid grape varieties by characterizing them chemically (tannic and anthocyanin profile, chromatic profile) with a focus on malvin in order to be able to compare them with wines from Vitis vinifera.

2 Materials and methods

2.1 Crossing and hybrid grape production

A total of 60 red hybrid grapes (HG) were considered in the present research (Table 1).

2.2 Red wine vinification

Winemaking process was carried out separately for each hybrid grape to obtain the corresponding monovarietal wines. Hybrid grapes were manually harvested at maturity during the 2019 and 2020 vintage. Grapes were crushed and destemmed the day of harvest. Potassium metabisulphite (3 g/hL) was added during the transfer of must to stainless steel tank. *Saccharomyces cerevisiae* was included to perform alcoholic fermentation at 23–25ºC.

Table 1. Hybrid grape varieties considered in the present research.

Color	Samples/Hybrids	N°
Red	Artaban Refart - chauff.	A1
	Artaban Liquart - chauff.	A2
	Artaban Solart - chauff.	A3
	Artaban 19	A4
	Artaban 566-19	A5
	Cabernet Cortis 2019	CC
	Cabernet Volos 20	CV1
	Cabernet Volos 570-19	CV2
	Merlot Khorus	K
	Monarch 521-19	Mo1
	Monarch 562-19	Mo2
	Monarch 609-19	Mo3
	Monarch 19	Mo4
	Prior 571-19	P1
	Prior 2020	P2
	Prior 605-20	P3
	Vidoc 580-19	V1
	Vidoc 594-19	V2
	Vidoc 603-19	V3
	Vidoc 19	V4
	Vidoc 604-20	V5
	Vidoc 606-20	V6
	3160-27-4 (from Fer Servadou)	X1
	3328-306 (from Marselan)	X2
	3328-168 (from Marselan)	X3
	Artaban 620-20	X4
	Artaban 621-20	X5
Rosé	Artaban Arclair	A6
	Artaban Armyl	A7
	Artaban Artiol	A8

Malolactic fermentation (MLF) was conducted in all cases at a maintained temperature of 22ºC and extended for a variable period of time depending on the hybrid grape.

Once the MLF concluded (malic acid content ≤0.2 g/L), wines were racked and directly bottled and stored at 16ºC until further analysis.

Wines from *Bouquet* varieties were provided by the experimental station *Domaine de Pech Rouge* from INRA (Gruissan, France). Chambre d'Agriculture de Gironde et Chambre d'Agriculture de l'Aude and Institut Coopératif du Vin (ICV) (Lattes, France) gave the rest of wines.

2.3 Total phenolic, proanthocyanidin, and anthocyanin analyses

Total polyphenol, proanthocyanidin, and anthocyanin contents of the 20 red monovarietal wines were spectrophotometrically determined in triplicate. Total phenolic content was measured according to a modified Folin Ciocalteu method to be applied in 96-well microplates [8] in a FLUO star Optima spectrophotometer (BMG LabTech, France). Results were expressed in mg of gallic acid equivalents/L wine.

Total proanthocyanidin and anthocyanin contents were estimated in triplicate through the Bate–Smith reaction [9] and the sodium bisulfite discoloration method [10], respectively, by using a V-630 UV–vis spectrophotometer (JASCO, Japan). Results were expressed in mg of catechin equivalents/L wine and in mg of malvidin equivalents/L wine, respectively.

2.4 HPLC analysis of anthocyanins

-Identification and quantification of anthocyanins were performed as previously described in the literature [11] by using a Thermo-Accela HPLC instrument. Results were expressed in mg of Mlv-3-O-monoglucoside/L wine.

-Malvidin-3,5-diglucoside – Malvin HPLC dosage

For malvin, an adapted HPLC method was necessary with a 1260 Infinity HPLC system coupled with a DAD detector and a 6460-triple quadrupole (QQQ) mass detector was used (Agilent, Waldbronn, Germany). The software used was the MassHunter Workstation (version B.08.00). The QQQ used a Dual AJS-ESI (Dual Jet Stream Electrospray Ionization) source, positive ionization mode with the parameters in Table 2. The system column was a Nucleosil 100-5C18 (25 cm × 4.0 mm, 5 µm). The following method was applied: flow rate of 1 mL/min, injection volume of 10 µL, detection wavelength at 520 nm. The solvents used were solvent A (water/formic acid 95:5 v/v) and solvent B (acetonitrile/formic acid 95:5 v/v) with the following gradient: solvent A: 0 min, 87%; 20 mins, 86%; 23 mins, 10%; 25 mins, 0%; 30 mins, 10%; 35 mins, 10%. The quantification was done with the help of ESI-SIM for the mass of the molecule (malvin:$C_{29}H_{35}O_{17}$, m/z: 655.578).

Table 2. Triple quad analysis parameters.

Parameters of QQQ	
Capillary voltage	4500 V
Fragmenter	135
Gas temperature	350 °C
Drying gaz	5 L/min
Nebulizer	50 psi
Temperature of gas sheath	250 °C
Flow of gas sheath	10 L/min
Acquisition margin	100-2000 m/z
Collision voltage fixed	8 V

-Malvidin-3,5-diglucoside – Malvin OIV dosage

Malvidin-3,5-diglucoside was analyzed in all samples according to the OIV method (OIV-MA-AS315-03, 2018). Briefly, 5 mL of twice diluted wine was added to 0.75 mL of ethanal solution (paraldehyde/absolute ethanol, 10:90 (v:v)) to treat the wine. After waiting for 20 minutes, 0.5 mL of treated wine is added to 1 drop of hydrochloric acid (37%) and 0.5 mL of sodium nitrite solution (10 g/L).

After 2 minutes and stirring, 5 mL of ammoniacal alcohol (absolute ethanol containing 5% concentrated ammonia) was added. After waiting for 10 minutes, the tube was centrifuged (5 minutes, 4500 rpm) then the supernatant was measured at 490 nm (T) relative to a solution of quinine sulphate (2 mg/L in acid sulfuric acid 0.1M). The result was expressed in mg of malvidin-3,5-diglucoside equivalent/L of wine [12].

C (mg of malvidin-3.5-diglucoside/L of wine)=(T-6)*0.49

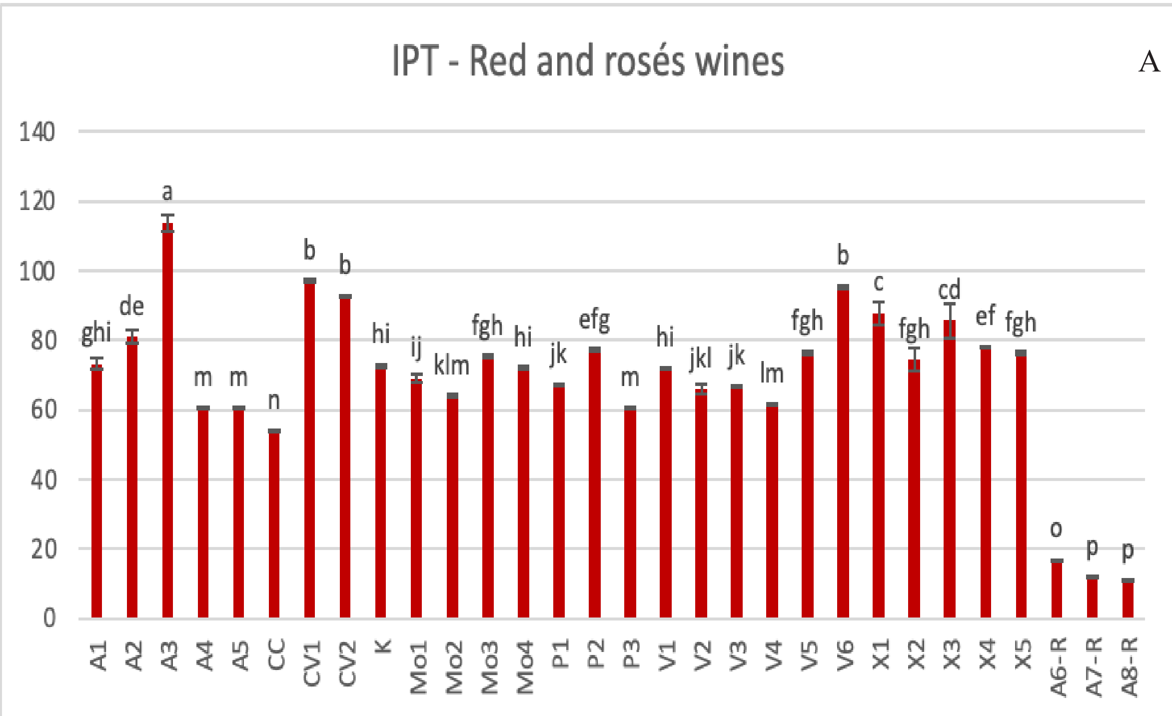
2.5 Statistical analysis

All the chemical analysis data (two bottles analyzed for each wine in triplicate, i.e. six repetitions for each sample) were statistically processed using XLSTAT (Version 2021.2.1, Excel 16.0.13929 (64bit), compatible with Windows 10). Normality was tested with the Shapiro-Wilk test and homoscedasticity with Levene's test. Then, parametric tests (ANCOVA and Tukey) were applied to test if the data are significantly different between them (p-value < 0.05).

3 Results and Discussion

3.1 Total phenolic, proanthocyanidin and anthocyanin contents

Total phenol index, proanthocyanidin and anthocyanin contents ranged, respectively, from 53.8 to 113,7, from 1.48 to 6.37 g Cat eq/L wine and from 98.7 to 1043.2 mg Mlv3glu eq/L wine (Fig. 1). Both phenolic and proanthocyanin values were highly consistent with those previously reported in the literature for monovarietal wines from common red grape varieties such as Cabernet Sauvignon, Merlot or Syrah [13−16]. Our results confirm the high tannin potential of most resistant varieties (especially Cabernet Volos) and many resistant varieties have higher levels. Meanwhile, anthocyanin results were generally greater than the bibliographic values for most of the wines considered.



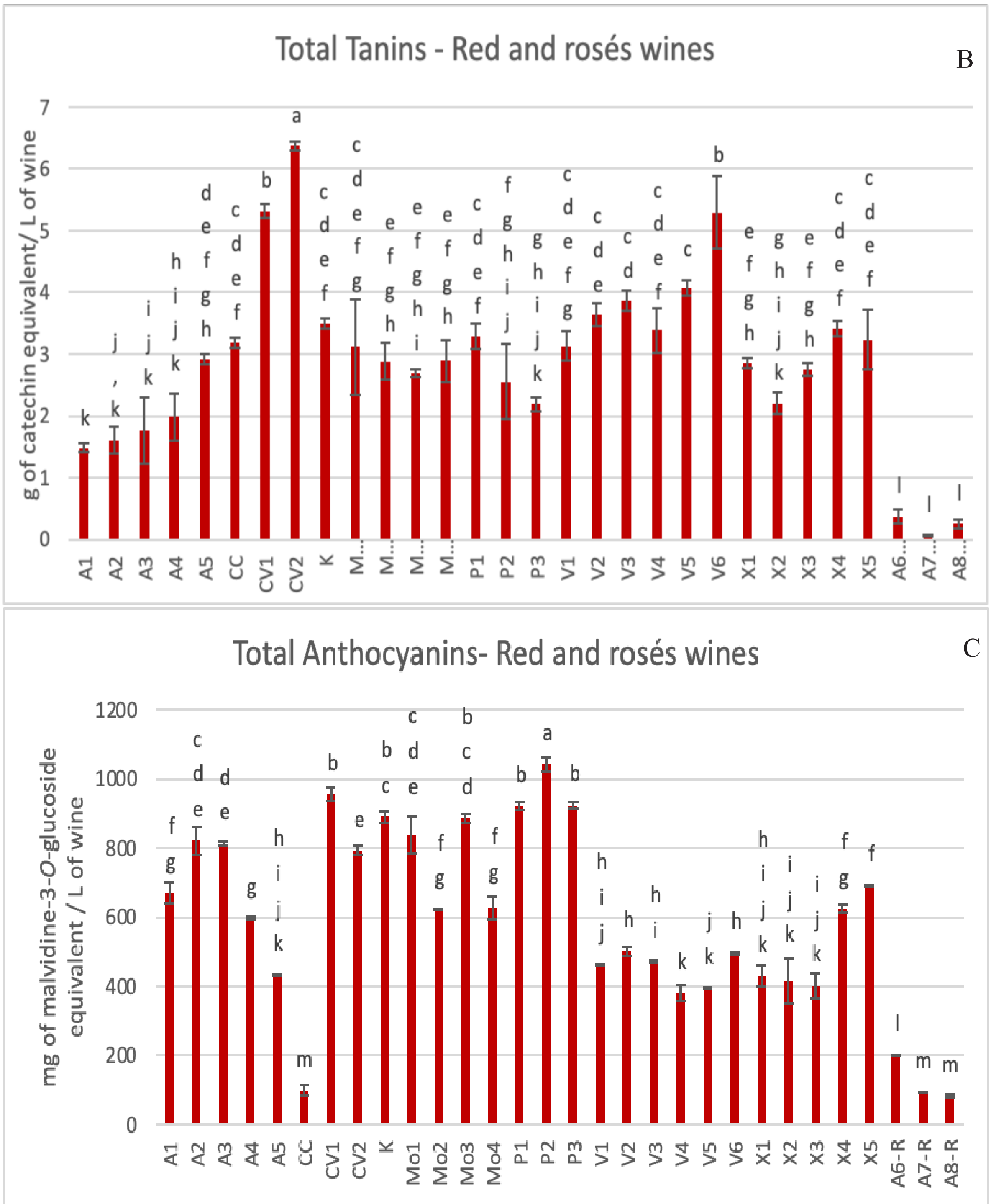


Figure 1. Total phenolics (A), total proanthocyanidins (B), and total anthocyanins (C) of monovarietal red wines elaborated with hybrid grape varieties. Lower case letters *a−g* show significant differences among hybrid grape varieties ($p < 0.05$).

3.2 Anthocyanin profile

The variability is high and neither vintage nor hybrid can play a significant role in grouping samples according to their concentration of molecular anthocyanins (Fig. 2). That said, malvidin-3-*O*-acetylglucoside is the majority anthocyanin for all samples except Vidoc wines and X1, X2, and X3. For the latter, the majority anthocyanin is malvidin-3-*O*-glucoside as is the case for Vitis vinifera wines and malvin is not detected.

Our results confirm for anthocyanins our findings of 2019 showing that the level of malvidin-3-glucoside appears to be linked to the % of vinifera genome in the varieties studied. Varieties containing less than 81% of vitis vinifera genome had anthocyanins mainly dominated by cyanidin [6]. The anthocyanin composition could be a criterion of wine quality (the black Bouquet varieties could present sensory characteristics superior to the other black resistant varieties when tasted).

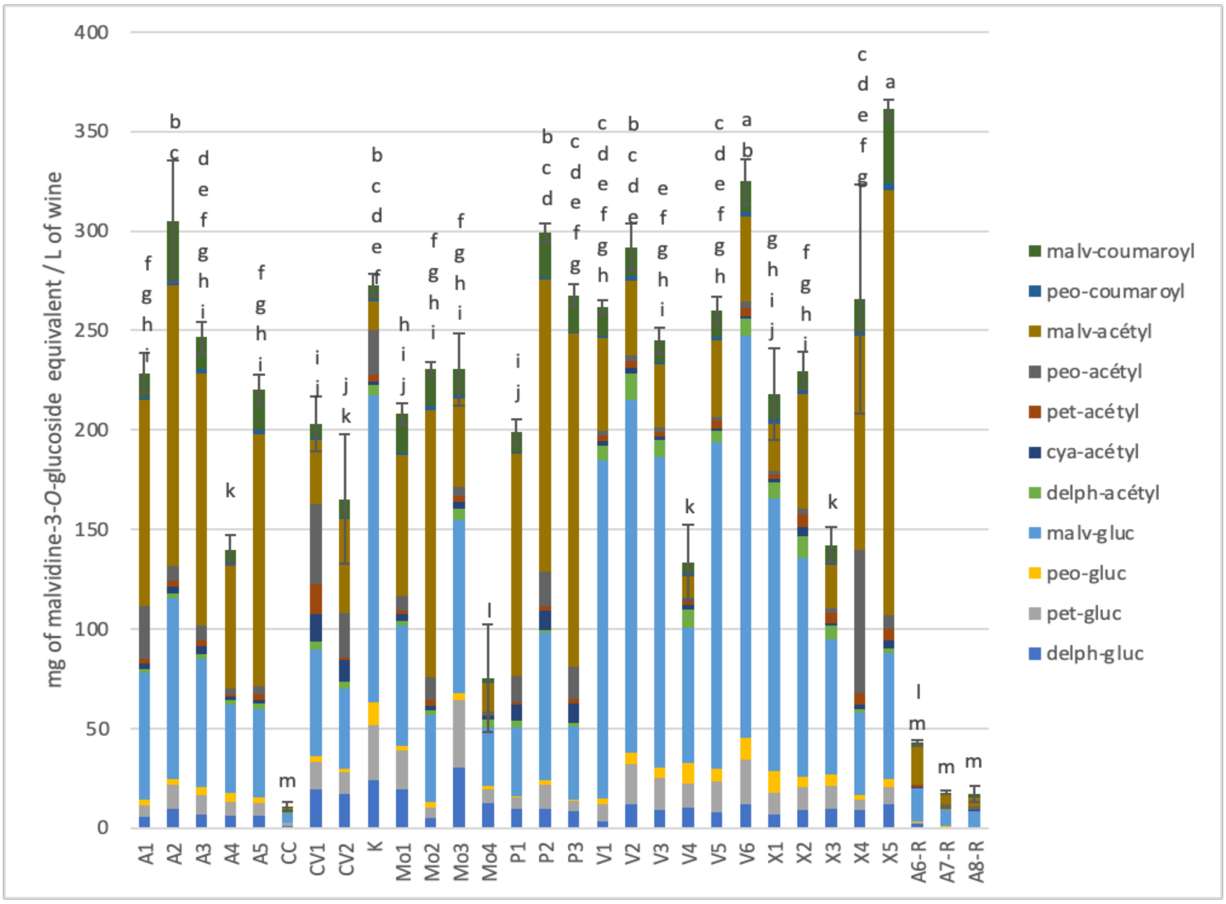


Figure 2. Anthocyanin profile of monovarietal red wines elaborated with hybrid grape varieties for delphinidine-3-*O*-glucoside, petunidine-3-*O*-glucoside, peonidine-3-*O*-glucoside, malvidine-3-*O*-glucoside, delphinidine-3-*O*-acetylglucoside, cyanidine-3-*O*-acetylglucoside, petunidine-3-*O*-acetylglucoside, peonidine-3-*O*-acetylglucoside, malvidine-3-*O*-acetylglucoside, peonidine-3-*O*-coumaroylglucoside, malvidine-3-*O*-coumaroylglucoside.

3.3 Malvidin-3,5-diglucoside (Malvin) analysis

In order to quantify malvin, the OIV method was applied to red and rosé wines. After treatment of the wine with an ethanol solution to combine the free sulfur dioxide, the molecule of interest oxidizes by adding nitrous acid and hydrochloric acid and at the end, we pass to a basic medium since the molecule there exhibits a bright green fluorescence. It is the latter that is then measured using a spectrophotometer [12].

Figure 3 shows the comparative results of this analysis between OIV and HPLC-DAD-QQQ methods. The maximum acceptable amount of malvin in wines must not exceed the limit of 15 mg/l of wine. However, it is obvious that all the samples for OIV method, except wine V4 with a concentration of 13.49 ± 1.05 mg/l, exceed this limit. The average concentration of malvin arrives at 30.12 ± 0.86 mg/l and V6 wine appears to have the highest concentration (53.7 ± 0.09 mg/l). This contrasts with the results obtained with our HPLC method, which shows that several hybrid wines including Vidoc and Cabernet cortis wines do not contain malvin. The OIV method gives results that can include other diglucosides from hybrid wines and not just malvin. In addition, rosé wines have a fairly high amount of malvin, a fact that does not justify their lower color (very few anthocyanins in total).

4 Conclusions

Regarding the content of total polyphenols, all of the wines also had normal concentrations as indicated in the bibliography [5, 6]. An interesting note is that Vidoc wines and X1 and X3 wines have, in general, five times more molecular tannins than other wines. In addition, the analysis of anthocyanins showed that the wines studied, except for the wines of Vidoc and the wines X1-X3, have a much higher concentration in comparison with the wines of Vitis vinifera. The concentrations of molecular anthocyanins seem to have quite high values and there is variability in the proportion of molecular anthocyanins for each wine. We confirmed that the level of malvidin-3-glucoside appears to be linked to the % of vinifera genome in the varieties studied. Varieties containing less than 81% of vitis vinifera genome had anthocyanins mainly dominated by cyanidin.

Numerous wines contain anthocyanin diglucosides, malvin having a very high concentration for certain wines. The Vidoc wines and the X1−X3, seem to present differences compared to the other wines, because they do not contain malvin and their main anthocyanin is malvidin-3-*O*-glucoside. All the other wines show a lower concentration of malvidin-3-*O*-glucoside and at the same time an irregularly high concentration of cyanidin-3-*O*-glucoside which co-elutes with malvin. The current OIV method gives different results for malvidol diglucoside compared to the use of our HPLC chromatography method (HPLC-DAD-QQQ). The current OIV method does not appear reliable in comparison with the HPLC-DAD-QQQ method for detecting malvine. The HPLC-DAD-QQQ method that appear to be a more precise and reliable method than that of the OIV method, and other studies will have to be carried out to better discern the anthocyanin content since the existence of anthocyanin diglucosides builds a complex background and to reliably differentiate Vitis vinifera wines from wines made from hybrids.

The continuation of analyzes by increasing the number of samples, of grape varieties and also by carrying out tastings of wines made from hybrid grape varieties must be carried out in order to compare their organoleptic quality with that of wines made from Vitis vinifera. The anthocyanin composition could be a criterion of wine quality.

Malvin analysis of red and rosé wines

	Malvin - OIV		Malvin - HPLC	
A1	22,18 ± 0,58	k,l,m,n	89,71 ± 14,83	de
A2	25,33 ± 0,06	i,j,k	102,31 ± 1,18	d
A3	22,47 ± 0,22	k,l,m	77,78 ± 4,63	ef
A4	18,32 ± 2,66	n,o	55,32 ± 2,61	g
A5	29,26 ± 0,22	g,h,i	30,02 ± 1,06	hi
CC	34,02 ± 0,39	e,f	nq	
CV1	35,67 ± 1,59	e	158,55 ± 2,25	b
CV2	49,51 ± 3,88	b	103,92 ± 3,31	d
K	41,25 ± 1,34	d	101,13 ± 0,88	d
Mo1	24,13 ± 1,22	j,k,l	101,74 ± 2,43	d
Mo2	19,25 ± 0,43	m,n,o	41,78 ± 1,39	gh
Mo3	28,16 ± 0,51	g,h,i,j	107,38 ± 3,33	d
Mo4	21,23 ± 0,10	l,m,n	60,67 ± 3,62	fg
P1	31,66 ± 0,20	e,f,g	132,21 ± 15,09	c
P2	41,44 ± 0,13	d	193,98 ± 17,70	a
P3	48,05 ± 1,53	b	135,71 ± 0,53	c
V1	21,23 ± 1,63	l,m,n	nq	
V2	18,17 ± 0,22	n,o	nq	
V3	15,35 ± 0,16	o,p	nq	
V4	13,49 ± 1,05	p	nq	
V5	42,23 ± 0,82	d	nq	
V6	53,70 ± 0,09	a	nq	
X1	19,89 ± 0,52	m,n	nq	
X2	19,00 ± 0,13	m,n,o	nq	
X3	19,04 ± 0,64	m,n,o	nq	
X4	40,28 ± 0,71	d	43,65 ± 1,78	gh
X5	43,88 ± 2,46	c,d	51,04 ± 0,81	gh
A6-R	47,62 ± 1,99	b,c	11,35 ± 1,44	i
A7-R	30,13 ± 0,01	f,g,h	nd	
A8-R	27,61 ± 0,42	h,i,j	nd	

Figure 3. Comparison of Malvin (Malvidin-3,5-diglucoside) in analysis of red and rosés hybrid monovarietal red wines elaborated with hybrid grape varieties. Nq : not quantify, nd: not detected.

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