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VALOVITIS, the value of minority or endangered vine varieties in the Pyrenean foothills

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Summary

The VALOVITIS project aims at improving the competitiveness of the wineries in the Pyrenean piedmont area through innovation and differentiation in a global market. During two consecutive harvests (2016-17), VALOVITIS monitored and characterised the agronomic behaviour of 20 grape varieties in the vineyards of the Germplasm Bank of the Government of Aragon in La Alfranca, Pastriz (Zaragoza). This work also assessed the technological and sensory properties of the wines produced following a basic winemaking protocol. Some of the varieties selected in this project, like the white variety 'Greta' and the red variety 'Parrel', showed a high potential to adapt to arid climates and to produce wines with a singular organoleptic profile.

K e y w o r d s : varietal heritage; climate change; VALOVITIS; market differentiation.

Introduction

The considerable heritage of vine genetic resources in the Pyrenean foothills is a valuable source of wine diversity. The current globalised winemaking market demands greater exploitation of genetic diversity, looking for singularity in flavours and valuing the terroir in which they are grown. Climate change and, more specifically, water scarcity and a progressive rise in average temperatures jeopardizes the sustainability, quality and competitiveness of the viticulture sector in the south of Europe. The continental Mediterranean climate prevails in Aragon, with cold winters and hot dry summers. However, the topography influences the patterns of this climate, ranging from the aridity of the Ebro valley to the permanent snow areas of the Pyrenees, through a succession of areas characterized by their diverse altitude, orientation or relief (Gobierno de Aragón 2007). These conditions lead to higher degrees of alcohol and falling acidity values in mature vineyards, which go against advisable winemaking conditions.

The drive towards worldwide standardisation of the markets has left behind traditionally exploited varieties which may be better adapted to new production conditions. The evaluation and preservation of these traditional varieties may provide small and medium size local wineries with a competitive advantage. Some of the key features considered valuable in this study are their agronomic yield, sensorial singularities and chemical characteristics which make these varieties well-adapted to face the effects of the climate change. The current rise in average temperatures leads to an increase in the sugar content in grapes. Thus, those varieties with lower sugar production capacity are of major interest in terms of ensuring sustainability of wine production in continental areas such as the Pyrenean foothills and Ebro Valley.

The initial selection of varieties considered for this study was based on popular knowledge about their agronomic and oenological behaviour. The baseline data and information was collected through an open participative approach (using the project website and direct contact with technical experts) as well as scientific works of identification of the Aragonese historical varietal heritage preserved in the Germplasm Bank of the Government of Aragon (Andreu *et al.* 2009 and 2010, Buhner-Zaharieba *et al.* 2010, Franco *et al.* 2015 and Losada *et al.* 2015). The molecular analyses in these works were carried out at the Experimental Station of Aula Dei by means of 8 microsatellite marker under project "Documentation, characterization and rationalization of grape germplasm prospected and conserved in Spain. Creation of a core collection (RF2012-00027-C05-02)" funded by the National Institute for Agricultural Research and Experimentation (INIA).

The Aragon Government's Agrifood Transfer Centre (CTA) and its Germplasm Bank, created in Movera (Zaragoza, Spain) in 1989, have identified and preserved vine vegetal material from 784 different entries in four different plots in the Ebro Valley. This bank currently has 219 varieties, 77 of them known but not common in Aragon, 75 minority varieties and 67 with unknown profiles, almost all of them absent from the commercial lists of authorised varieties in Spain. The main purpose of the bank is the mitigation of biodiversity loss, working on prospections and the preserva-

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tion of ancient varieties close to disappearing in the region of Aragon (northeast Spain). It aims to safeguard valuable genetic material for the future that may help the sector face unexpected market fluctuations and/or the adverse consequences of climate change.

The project Interreg POCTEFA VALOVITIS (www.valovitis.eu), co-funded by the European Regional Development Fund (ERDF), is aimed at valuing the historical varietal heritage of vines in the Pyrenean foothills area in France and Spain. Local wineries need to be able to differentiate their products in order to obtain a substantial commercial advantage and improve their competitiveness, particularly in export markets. VALOVITIS has worked on the identification and evaluation of unknown or endangered varieties that may meet market requirements while also possessing the necessary capacity to adapt to climate change. Specialised technicians have been working on the initial steps of medium and long-term approaches to place these minority varieties at the service of the wine sector. During the 2016 and 2017 harvests, the Agrifood Research and Technology Centre of Aragón (CITA), endorsed by CTA, characterised 20 varieties through agronomic monitoring of the winemaking process and a sensorial analysis of the wines produced from the two Germplasm Bank vineyards located in La Alfranca, Pastriz (Zaragoza). The volatile composition profiles of the wines were also thoroughly monitored through chemical quantitative analysis of all the relevant families of compounds involved in the wine aroma. Special attention was paid to those related with varietal aromas.

Material and Methods

Plant material: The minority varieties selected came from 25 different locations all around the region of Aragon. Some of them were found in more than one location (Tab. 1). The prospection was more intense around the Ebro Valley and in the Pyrenean foothill areas. Identification in prospection fieldwork was based on sampling, recording and molecular characterisation at the Institute Français de la Vigne et du Vin in Montpellier using a polymerase chain reaction (PCR) technique that considers 23 markers.

Table 1

Varieties included in the Interreg POCTEFA VALOVITIS project

	Name	Synonym	GB Code	Genotype N°	Berry	Wine code	Location	Geographical region of origin
1	Greta		ARAG-66-20	MEXT 1957	White	6B1; 7B1	Camporrotuno Oncins	Pyrenees
2	Unknown		ARAG-66-29	MEXT 1962	White	6B2; 7B2	Ayerbe	Pyrenees
3	Jarrosuelto		ARAG-25-09	MEXT 0004	White	6B3; 7B3	Arándiga Cariñena Fuendejalón Paniza	Ebro Valley
4	Unknown		ARAG-64-32	MEXT 1952	White	7B12	Gavín	Pyrenees
5	Albana	Pasa Valenciana	ARAG-03-12	GEN 0520	White	6B4; 7B4 7B4_1; 7B13 7B14; 7B15	Alcubierre Casbas La Almolda	Ebro Valley
6	Rotuna		ARAG-62-15	MEXT 1948	White	6B6; 7B6	Aínsa	Pyrenees
7	Olivana		ARAG-65-15	MEXT 1954	White	6B7; 7B7	Oliván	Pyrenees
8	Tortozón		ARAG-61-23	GEN 0150	White	6B9; 7B9	Monlora	Prepyrenees
9	Beturian		ARAG-61-30	MEXT 1947	Red	6T6; 7T6	Aínsa	Pyrenees
10	Unknown		ARAG-62-16	MEXT 1949	Red	6T7; 7T7	Aínsa	Pyrenees
11	Unknown		ARAG-20-10	MEXT 1847	Red	6T18; 7T18	Villanueva de Huerva Zaragoza	Ebro Valley
12	Ambrosina	Aubun	ARAG-12-03	GEN 0906	Red	6T2; 7T2 6T12; 7T12 6T32	Ainzón Almonacid de la Sierra	Ebro Valley
13	Unknown		ARAG-64-19	MEXT 1951	Red	6T8; 7T8 6T22; 7T22	Biescas	Pyrenees
14	Tortozona Tinta		ARAG-28-06	MEXT 0031	Red	6T23; 7T23 6T25; 7T25	Almudevar Muniesa	Ebro Valley
15	Salceño		ARAG-66-15	MEXT 1958	Red	6T24; 7T24	Camporrotuno	Pyrenees
16	Unknown		ARAG-25-13	MEXT 1863	Red	6T30; 7T30	Castejón de Monegros	Ebro Valley
17	Morate		ARAG-23-12	GEN 0063	Red	6T31; 7T31	Paniza Villarroya de la Sierra	Ebro Valley
18	Unknown		ARAG_01_07	MEXT 1920	Red	6T5; 7T5	La Almolda	Ebro Valley
19	Grumel		ARAG-65-21	MEXT 1956	Red	6T9; 7T9	Camporrotuno El Pueyo de Aragüás	Pyrenees
20	Parrel		ARAG-12-02	GEN 0905	Red	6T10; 7T10_1; 7T10_2	Ainzón Sástago	Ebro Valley

GB Code: Germplasm Bank Code;
Genotype Nº: code assigned by IMIDRA (Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario) to the genotype.

Agronomic and oenological evaluation: The VALOVITIS project monitored and characterised 20 varieties (Tab. 1) during two consecutive harvests (2016-17) in the vineyards of the Germplasm Bank (Government of Aragon). This study includes the information obtained in the two espalier vineyards located in La Alfranca, Pastriz (Zaragoza). They are in the same area and managed simultaneously with the same practices. The vines are grown in groups of 4 and 5 vines respectively and are available between 10 to 50 vines per variety in each plot. Both vineyards have irrigation facilities for use when required depending on the weather conditions.

The project produced 19 white wines from 8 different varieties and 32 red wines from 12 varieties (Tab. 2). Some of the varieties are known but rarely cultivated and others are unknown and, in some cases, only grown in the Aragonese region.

Table 2

Summary of the wines and varieties assessed during VALOVITIS project in the vineyards of La Alfranca (Germplasm Bank of the Aragon Government)

Harvest	Entries to the Germplasm Bank	White varieties	White wines	Red varieties	Red wines
2016	47	7	7	12	16
2017	49	8	12	12	16

The wine-making took place in an underground wine cellar with a thermal range of 12 to 14 °C situated in Almonacid de la Sierra (Zaragoza). Quantities of grapes ranged between 3 and 100 Kg placed in stainless steel tanks of 25, 15, 10 and 5 L capacity.

The agronomic evaluation started with the field monitoring of grape maturity based on the sugar content, acidity and pH levels. Yields were assessed during the harvest. The date of harvest, number of vines, number of bunches and total weight per variety were recorded.

Once the grapes were duly identified and stored in boxes of 20 kg, they were transferred to the winery where a registration protocol was used to record the average weight of the bunches, number of grapes per bunch and weight of 100 grape seeds.

Technical experts identified the grapes of every variety and measured the brix (estimated alcohol content), total acidity and pH in the must, prior to vinification. The operations carried out in the winery included destemming, squeezing and dosing with 50 $mg \cdot kg^{-1}$ of sulphur dioxide. The white wines were vinified after a direct pressing and the red wines underwent a seven-day maceration with daily lees stirring. The selected dry yeast used was *Saccharomyces cerevisiae*. The fermentation temperature in whites did not exceed 13 °C and in reds 22 °C. Once the alcoholic fermentation was finished, the wine was racked and sulphur dioxide was added at a rate of 50 $mg \cdot L^{-1}$. In the red wines, malolactic fermentation was not performed.

The traceability of each vinification operation was monitored and dated, including debourbage, start and finish of fermentation, racking, filtration and bottling.

Chemical characterization of wines: During the bottling process, the wines were sampled and analysed in the laboratory. The parameters measured were alcohol content at 20 °C by infrared absorption, pH using potentiometry, malic acid by an enzymatic method (LD: 0.0, LQ: 0.1), colour intensity by UV-VIS spectroscopy and the total polyphenol index by absorbance at 280 nm. Flow injection was used to analyse the content of reducing sugars, total and volatile acidity and free sulphur dioxide.

Organoleptic characterization: Eleven trained testers evaluated the sensory parameters of the wines using specific descriptors of aroma and taste. In the case of white wines, the testers assessed thiolic, terpenic, phenolic and fermentative characteristics. The descriptors for red wines were vegetal flavours and thiolic, terpenic, fermentative, floral, vegetal and spicy notes.

Chemical quantitative analysis of volatile compounds: Several families of volatile compounds were analysed in the wines using five different analytical methods for a total of more than 80 different volatiles. The quantitative analysis of major compounds was carried out using a validated and published method based on Gas Chromatography with Flame Ionization Detection (GC-FID) (Ortega *et al.* 2001). Minor volatile compounds were analysed with Solid Phase Extraction (SPE) combined with Gas Chromatography with Mass Spectrometry Detection (GC-MS) (Lopez *et al.* 2002). Polyfunctional mercaptans were analysed with a method based on derivatisation followed by SPE and Gas Chromatography with Negative Chemical Ionization Mass Spectrometry Detection (GC-NCI-MS) (Mateo-Vivaracho *et al.* 2010). Alkylmethoxypyrazines were analysed following a validated method based on Stir Bar Sorptive Extraction (SBSE) GC-MS (Wen *et al.* 2018). Rotundone analysis was carried out using a previously published method based on SPE-GC-MS (Culleré *et al.* 2016).

Results and Discussion

During the evaluation of the vines, grown in the same area under equal conditions, the varietal character was revealed when characterizing the type of wine obtained from a chemical and agronomical point of view (Fig. 1). The PCA figure shows the closeness of wines elaborated with the same variety. In comparison the season-to-season variation is less relevant as variations in production, brix and total acidity are not enough to group the wines by year.

Among the 20 varieties prospected and assessed, a selection was made considering the results obtained from the chemical and agronomic characterization of the wines. A total polyphenol index above 30 and a colour index above 5 were the main selection criteria in the case of red wines. High acidity and low alcohol content were the key features for the white wines.

Using these criteria, the VALOVITIS project identified 7 varieties of major interest due to their commercial implementation potential; 4 whites: 'Greta', 'Albana', 'Rotuna' and 'Olivana', and 3 reds: 'Parrel' (Franco *et al.* 2018), 'Beturian', and 'Ambrosina', which is the local name for

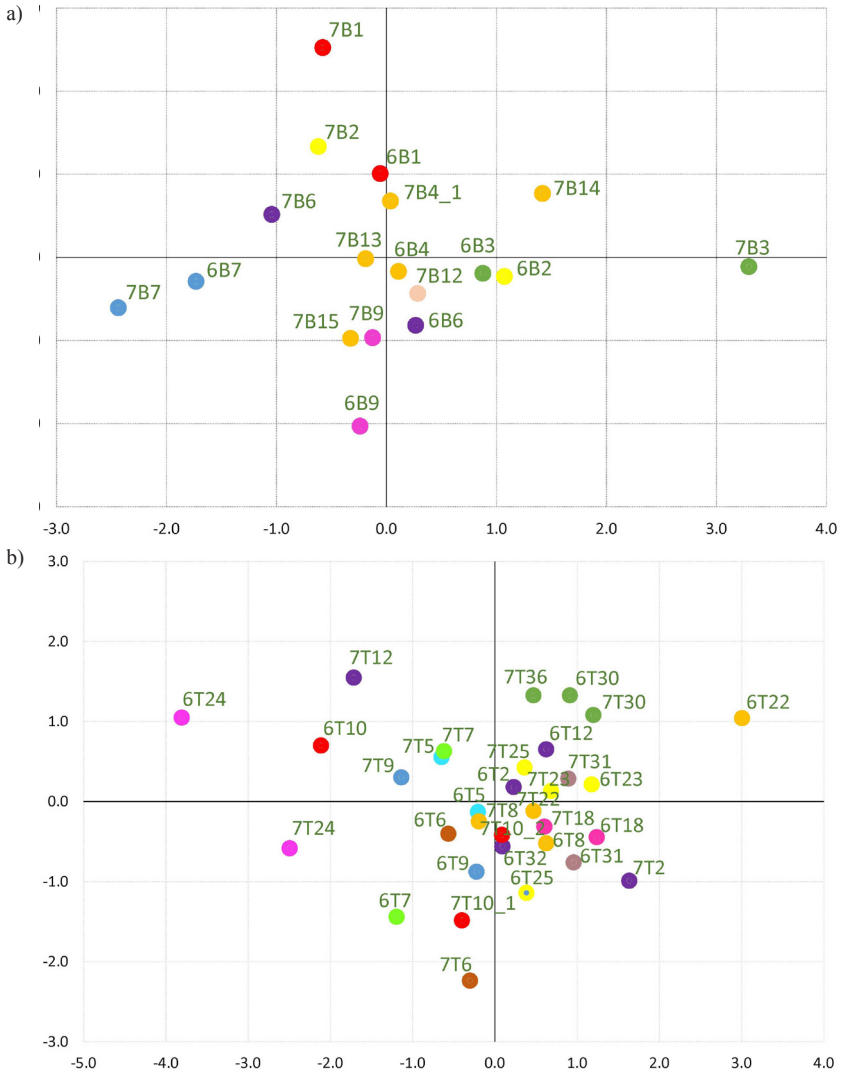

Fig. 1: Principal component analysis (PCA) based on production, brix and total acidity records in all white (**a**) and red (**b**) wines produced in 2016 and 2017. Varieties classified by colour. Wine code: year (6: 2016 or 7:2017) – type of wine (B: white; T: red) – Nr. vinification).

Aubun, a variety found also in La Rioja and Navarra. In the case of 'Ambrosina', two different wines were produced distinguishing the origin of the material evaluated, wines 6T2 and 7T2 ('Ambrosina' 1) were obtained from vines from Ainzón and 6T12 and 7T12 ('Ambrosina' 2) from Almonacid de la Sierra. Olivana is an exception to the selection criteria explained above because it presents high sugar content, but it is combined with high acidity values, what makes this variety unique and interesting for obtaining alternative products such as sweet wines. Only the results obtained for these seven selected varieties are presented from here.

These varieties produced substantial yields that in some cases exceed the limits allowed by certain Protected Designation of Origin criteria.

Agronomic and chemical assessment: Based on analytical data, a Principal Component Analysis (PCA) of the white and red wines made from the selected varieties identified the analytical parameters that grouped the wines by common patterns. The parameters considered in white wines were pH, total acidity, estimated alcohol content, potassium content, total polyphenol index (TPI) and malic acid content. The assessment of red wines also included colour intensity (Fig. 2). In the case of white wines, two factors explained 78.6 % of the variance, while for the reds, three factors were needed to explain 90.9 %. It is important to highlight that, in the case of red wines, potassium, pH and malic acid grouped, contributing to factor 1 and explaining 43.8 % of the variance. The chemical profile of the wines (Tab. 3) show that 'Albana' and 'Greta' produced white wines with greater malic acid and lower alcohol content, while the 'Olivana' wines had less malic and a greater alcohol content.

In the case of red wines, the 'Beturian' variety produced wines with higher colour intensity in 2017 and TPI with a sufficient alcohol content. The difference in colour intensity between 2016 and 2017 within 'Beturian' could only be explained by a mechanism not related with the variety or the vintage, like the combination of anthocyanins with acetaldehyde at the end of the alcoholic fermentation. The 'Parrel' and 'Ambrosina' varieties also had a good alcohol content and colour intensity.

Organoleptic assessment: The sensory profile patterns of all the red wines was practically replicated in the two harvests (Fig. 3). The red wines had a medium-high aromatic intensity, emphasizing their terpenic, spicy and fermentative character. In terms of taste, the aromatic intensity and acidity stood out, observing an almost total absence of

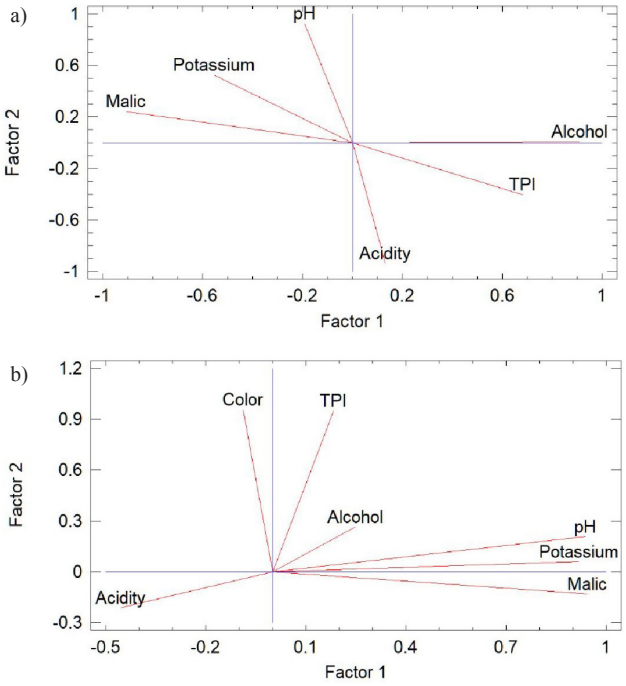

Fig. 2: Plots of factor loadings (PCA analysis) in white (**a**) and red wines (**b**) made from selected varieties in 2016 and 2017. TPI: Total Polyphenol Index; Alcohol: Alcohol degree.

the tannic descriptors. A PCA statistical analysis performed separately for white and red wines to identify descriptors of the aroma enabled the wines to be grouped according to their aromatic characteristics. The analysis revealed that three factors explained 87.8 % of the variance for white wines and 86.3 % for red wines.

As with the PCA of the chemical parameter values described above, the descriptors of the aroma also grouped the white wines by thiolic and terpenic character against fermentative and vegetal in the case of white wines. The red wines were grouped according to their terpenic, phenolic and spicy flavours against thiolic, floral and vegetal notes. In this statistical assessment, once more, the aroma descriptors grouped wines of 2016 and 2017 harvests by varieties, leaving the influence of the harvest conditions in second place.

The 'Albana' white wines had a stronger fermentative character, while 'Greta' was more thiolic and terpenic. In the case of red wines, the 'Parrel' variety produced more terpenic wines and those from 'Ambrosina' had a more fermentative and floral character (Fig. 3).

Volatile chemical composition: Tab. 4 shows the concentration of some selected compounds representative of different chemical families of aroma compounds with varietal origin. Varietal thiols (3-mercaptohexanol and its acetate) related with tropical fruit notes were found above their olfactory thresholds in some of the wines. The levels of 3-mercaptohexanol were remarkably high in the 2017 'Beturian' wine, while the levels of 3-mercaptohexylacetate in the 2016 'Albana' wine were comparable to those found in 'Sauvignon' wines (Mateo-Vivaracho *et al.* 2010). Rotundone, a positive compound for wine aroma, was not found above its sensory threshold in any of the wines. As expected for non-terpenic varieties, the concentration of linalool was well below the levels found in muscat varieties. Fortunately, none of the wines showed large quantities of TDN or alkylmethoxypyrazines, all detrimental compounds for wine aroma.

Conclusions and future prospects

Due to limitations in the availability of vegetal material, it was not possible to conduct a complete assessment of season-to-season variations, however, the varietal effect was noticiable over the growth conditions for the two harvests in terms of both analytical and organoleptic data. The varietal typicity of the wines should be confirmed extending the

Table 3

Performance and chemical analysis of wines made from selected varieties

Wines and harvest	Variety	Wine code	Yield ($kg \cdot vine^{-1}$)	Alcohol degree (v/v)	Total acidity ($g \cdot L^{-1}$) TH_2	pH	Malic acid ($g \cdot L^{-1}$)	Potassium ($g \cdot L^{-1}$)	Colour intensity	Total polyphenolic index
White wines - 2016	Greta	6B1	6.10	11.61	5.00	3.53	1.50	0.73	0.12	5.40
	Albana	6B4	4.12	12.96	5.10	3.43	1.10	0.70	0.06	5.70
	Rotuna	6B6	2.86	13.61	6.60	3.21	0.90	0.57	0.16	5.40
	Olivana	6B7	7.44	15.57	6.80	3.11	0.80	0.50	0.15	6.30
White wines - 2017	Greta	7B1	9.98	10.84	5.50	3.38	1.70	0.89	0.06	4.40
	Albana	7B4	6.02	11.98	6.20	3.39	1.80	0.89	0.07	4.30
	Rotuna	7B6	7.27	12.99	6.40	3.18	1.20	0.79	0.09	5.40
	Olivana	7B7	8.03	15.27	6.70	3.27	1.00	0.71	0.09	5.70
Red wines - 2016	Ambrosina 1	6T2	5.16	15.93	9.40	3.32	1.20	0.88	10.97	33.60
	Ambrosina 2	6T12	4.50	14.75	6.40	3.67	0.90	1.05	7.74	53.20
	Beturian	6T6	3.32	12.09	6.90	3.30	0.80	0.80	7.42	37.10
	Parrel	6T10	11.72	13.29	5.90	3.60	1.30	0.96	12.43	39.60
Red wines - 2017	Ambrosina 1	7T2	5.29	15.14	6.30	3.21	0.60	0.79	5.29	34.30
	Ambrosina 2	7T12	12.23	13.42	5.00	3.60	1.60	1.28	5.62	24.60
	Beturian	7T6	2.16	14.40	6.40	3.36	0.80	1.15	18.75	49.00
	Parrel	7T10	5.29	13.86	5.40	3.78	1.80	1.38	8.40	38.20

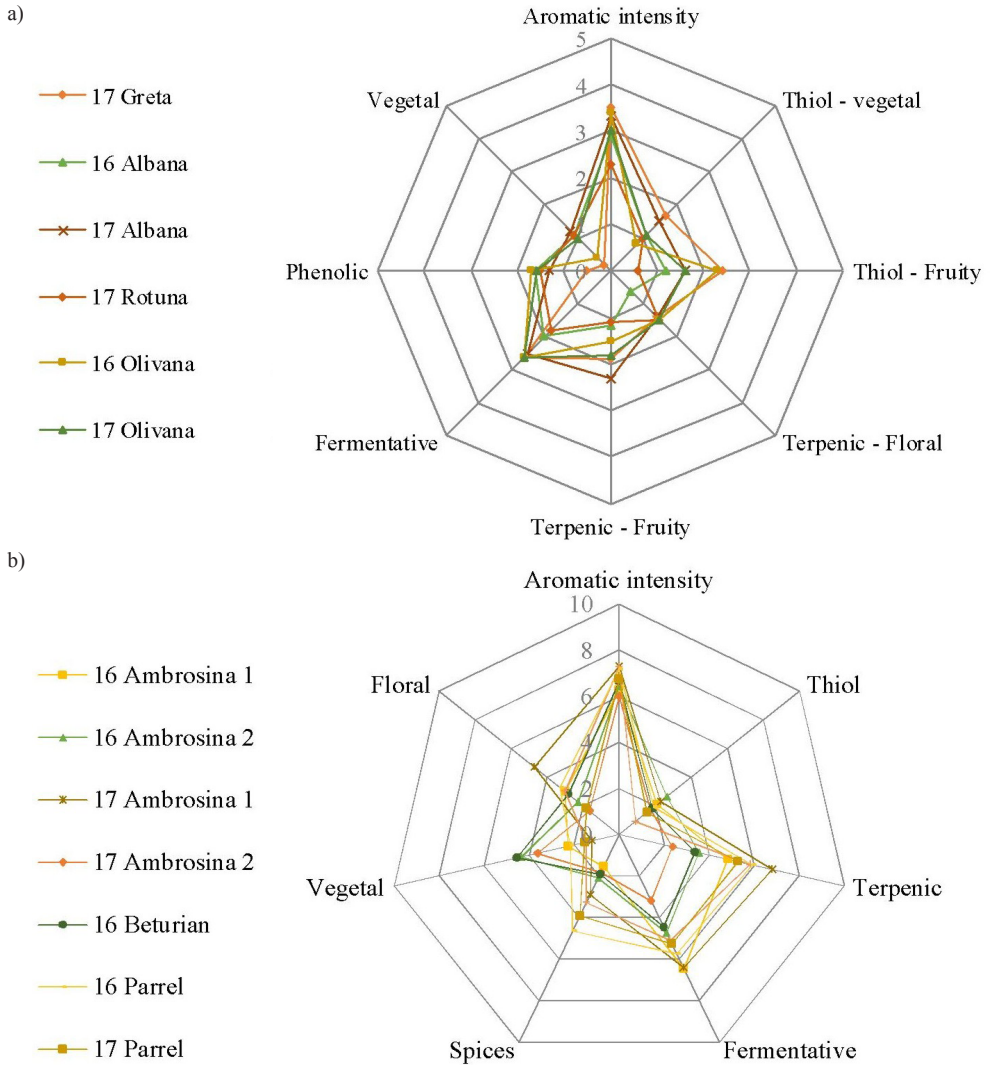

Fig. 3: Radial diagram of sensorial descriptive values of VALOVITIS white (**a**) and red (**b**) wines made from selected varieties. White wines values range from 0 to 5 and red wines 0 to 10.

Table 4

Concentration in ng·L^{-1} of selected varietal volatile compounds determined in wines made from selected varieties

Wines and harvest	Variety	Wine code	3-mercapto-hexanol	3-mercapto-hexyl acetate	Rotun-done	IPMP	IBMP	β-damas-cenone	TDN	Linalool
	Olfactory threshold (ng·L^{-1})		60	4	16	2	15	50	2000	25000
White wines -2016	Greta	6B1	<L.Q.	55.4	<L.Q.	<L.Q.	<L.Q.	3415	<L.Q.	3971
	Albana	6B4	23	718.5	<L.Q.	<L.Q.	<L.Q.	7016	<L.Q.	5206
	Rotuna	6B6	<L.Q.	35.4	<L.Q.	<L.Q.	<L.Q.	3975	<L.Q.	5578
	Olivana	6B7	<L.Q.	32.3	<L.Q.	<L.Q.	0.29	4466	<L.Q.	4409
White wines - 2017	Greta	7B1	<L.Q.	6.1	3.48	0.27	0.32	2065	682	2750
	Albana	7B4	164	21.2	<L.Q.	0.78	0.52	5955	579	3591
	Rotuna	7B6	152	12.5	1.11	0.95	0.53	1865	675	6458
	Olivana	7B7	141	10.6	<L.Q.	0.65	0.74	1142	655	3234
Red wines - 2016	Ambrosina 1	6T2	14	161.5	<L.Q.	<L.Q.	0.19	3286	<L.Q.	3677
	Ambrosina 2	6T12	<L.Q.	81.2	<L.Q.	<L.Q.	0.16	2700	<L.Q.	4052
	Beturian	6T6	<L.Q.	217.7	<L.Q.	<L.Q.	0.43	2773	<L.Q.	3663
	Parrel	6T10	<L.Q.	294.0	1,35	<L.Q.	0.89	1101	<L.Q.	2173
Red wines - 2017	Ambrosina 1	7T2	<L.Q.	97	1.95	1.31	0.95	5095	1091	4506
	Ambrosina 2	7T12	<L.Q.	5.7	2.04	2.04	0.75	3484	419	3052
	Beturian	7T6	1147	7.7	<L.Q.	0.82	1.16	6678	1823	4396
	Parrel	7T10	330	8.8	3.63	3.63	2.24	2361	1337	3863

<L.Q.: concentration under the limit of quantification of the method; IPMP: 3-isopropyl-2-methoxypyrazine; IBMP: 3-isobutyl-2-methoxypyrazine; TDN: 1,1,6-Trimethyl-1,2-dihydronaphthalene.

study in the following years. Plant material coming from the Ebro valley performed well in warm and dry conditions. Meanwhile, the varieties obtained from northern areas (wetter and colder regions) showed desirable qualities to compensate for the effects of climate change in wine production. Among the varieties selected in the VALOVITIS project, some such as 'Greta' demonstrated a high capability of adapting to more arid environments, thus revealing their value in the face of global warming. Others such as 'Olivana' had good production yields while their alcohol content remained high, making them suitable for obtaining alternative products such as sweet wines.

Aragón possesses a valuable varietal heritage. It is worth continuing to work on its preservation and to provide valuable tools enabling the local wine sector to offer uniqueness and differentiation in the markets. The white varieties 'Albana', 'Greta' and 'Rotuna' stand out. In the case of the red varieties, 'Ambrosina', 'Parrel' and 'Beturian' are of greatest interest.

The transfer of this knowledge to wineries and professionels in the sector can provide a competitive advantage to small and medium enterprises in the local wine industry, maintaining economic and social dynamics in the rural areas covered by the project.

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