

Capturing yeast associated with grapes and spontaneous fermentations of the Negro Saurí minority variety from an experimental vineyard near León

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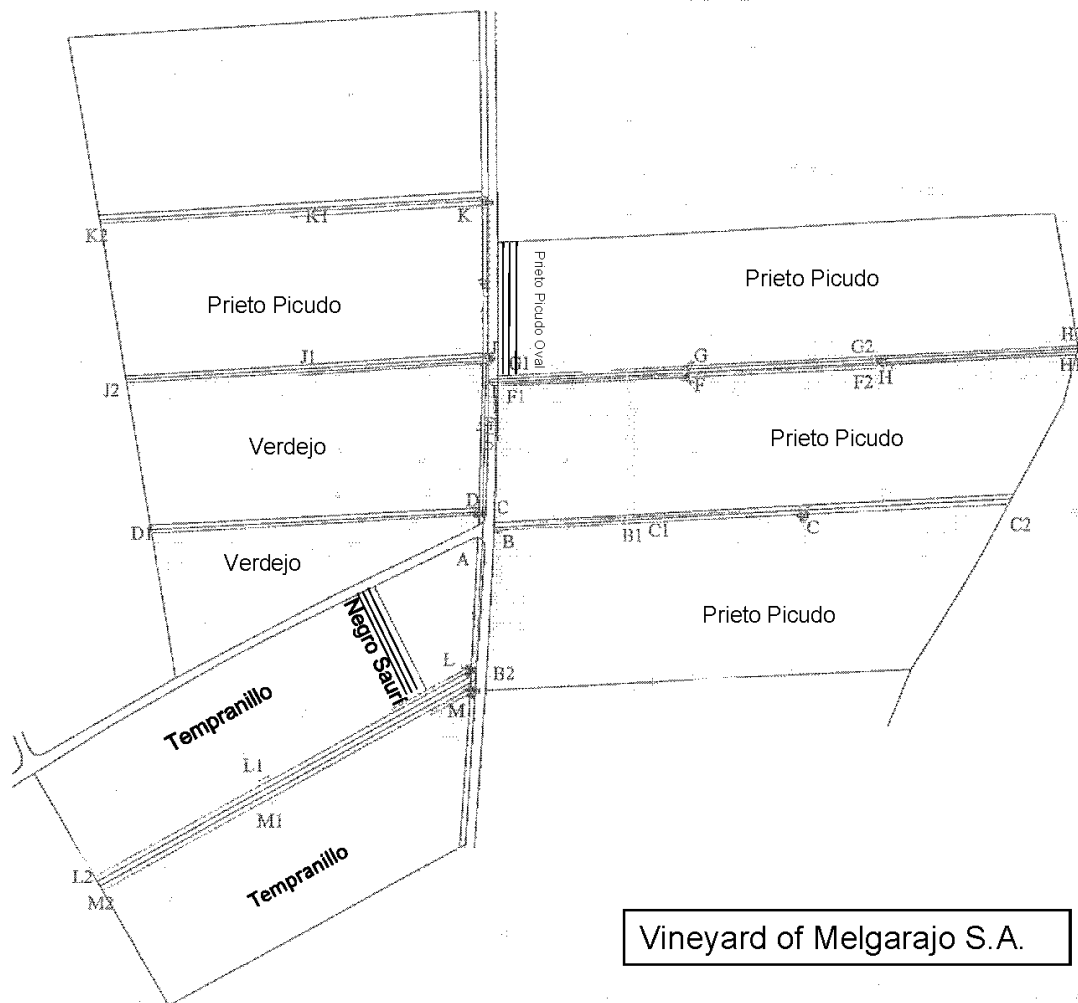
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Supplementary Fig. S1. Map of experimental vineyard at at Melgarajo S.A.



L1 Negro Sauri (3 rows, 15 cultivars; 300 plants (used in the study). Tempranillo grapes are sold.

M1 Tempranillo (grapes are sold)

D and E Verdejo (commercial wine is made off-site and not in experimental winery)

B, F, G and K Prieto picudo (commercial wine is made off-site and not in experimental winery)

G Prieto Picudo oval (clonal research).

An empty plot (behind K) to be planted with clones (from ITACYL's Negro Sauri Research) for future wine production

Supplementary Table S1. Average temperature (Tm; °C) and precipitation (P; mm) for the period 2014-2017 (October 2013 - September 2017) as registered at the meteorological station of Mayorga de Campos, Valladolid.

	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Ave
Tm 2014	12.4	5.8	2.0	5.5	5.0	7.8	12.3	13.5	18.1	19.8	19.7	18.1	11.7
Tm 2015	14.6	8.5	3.4	1.6	3.7	8.1	10.9	14.7	19.4	22.7	20.2	15.4	12.0
Tm 2016	11.8	8.0	4.9	5.4	5.2	5.9	8.4	12.3	18.3	22.3	21.6	17.7	11.8
Tm 2017	12.8	6.5	3.8	2.3	6.3	8.2	11.4	15.7	21.3	21.6	20.8	16.2	12.3
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Total
P 2014	82.2	11.5	60.0	58.6	63.6	21.8	33.3	22.4	10.3	26.8	1.2	38.6	430
P 2015	4.0	89.6	26.3	33.7	14.5	9.0	53.8	23.1	121.2	17.1	2.8	37.4	432
P 2016	84.2	42.2	22	105.2	47.4	31.6	106.4	78.4	2	1.2	3.6	11	535
P 2017	29.2	22.8	21	20.4	56.4	13.6	7.87	30.9	22.21	14.54	15.15	2.63	257

Supplementary Table S2. Global MCA: eigenvalues, cluster selection, biotypes, strains and distances

GLOBAL MCA: 110 strains and CROSS strain (supplementary) – 22 gel bands					
TABLE 1: EIGENVALUES AND RETAINED FACTORS FOR CLUSTERING 9 factors - 64,5 %			TABLE 2: CLUSTER SELECTION highest Calinski-Harabasz criterion; smallest Davies-Bouldin's index		
Factor	Variance of the Factor (eigenvalue)	% of explained variance	Cumulated % of explained variance	Benzécri's modified rates (%)	Retained according to Kaiser's criterion
1	0,110	11,0	11,0	40,5	X
2	0,106	10,6	21,5	35,4	X
3	0,076	7,6	29,1	9,0	X
4	0,071	7,1	36,2	6,3	X
5	0,068	6,8	43,0	4,8	X
6	0,061	6,1	49,1	2,5	X
7	0,056	5,6	54,7	1,0	X
8	0,053	5,3	59,9	0,5	X
9	0,046	4,6	64,5	0,0	X

Criteria	5 clusters	6 clusters	7 clusters
Intra-cluster inertia	0,392	0,350	0,309
Inter-cluster inertia	0,254	0,295	0,337
Explained inertia (%)	39,302	45,751	52,160
Calinski-Harabasz (pseudo F) criterion	16,997	17,542	18,717
Davies-Bouldin's index	1,385	1,203	1,052

TABLE 3: 7 biotypes, strains and distances					
I		III		III continuation	
Strain	Distance	Strain	Distance	Strain	Distance
1415	0,277	3416	0,234	1416	0,392
4116	0,299	3616	0,234	1714	0,392
2117	0,34	4016	0,234	2315	0,410
4314	0,392	4216	0,234	2014	0,410
2217	0,465	4316	0,234	3917	0,414
3914	0,5	916	0,263	5116	0,429
1817	0,617	1016	0,263	3116	0,432
1617	0,623	2116	0,263	3816	0,458
2417	0,687	3717	0,280	3916	0,458
3317	0,687	3817	0,280	2415	0,458
2414	0,712	4117	0,280	4617	0,494
3217	0,75	2716	0,280	4717	0,494
2614	0,813	2816	0,280	2016	0,522
3614	1,145	3016	0,280	3617	0,553
CROSS	1,373	3716	0,280	4017	0,575
		5016	0,280	2115	0,577
		2515	0,289	1616	0,633
		2216	0,292	3216	0,993
		5216	0,292	1716	0,998
		3114	0,319	1816	0,998
		2916	0,340	2114	1,055
		2616	0,353	1715	1,635
		3517	0,353	3516	1,863
		4416	0,368		

II		IV		VI		VI continuation	
Strain	Distance	Strain	Distance	Strain	Distance	Strain	Distance
2814	0,192	4317	0,774	4516	0,148	3214	0,513
3014	0,192	4417	0,574	1915	0,148	1515	0,519
		4517	0,778	2015	0,148	2416	0,595
		4817	0,702	1514	0,148	1115	0,595
		1116	0,537	1614	0,148	4716	0,607
		1216	0,511	1916	0,241	1814	0,765
		1316	0,653	2316	0,241	4214	0,782
		1516	0,587	415	0,241	4014	1,031
		2214	0,472	615	0,241		
				1215	0,241		
				1315	0,241		
				2714	0,241		
				2914	0,241		
				3314	0,241		
				3414	0,241		
				2215	0,288		
				2516	0,299		
				3316	0,310		
				4616	0,333		
				4816	0,333		
				4916	0,333		
				2514	0,454		
				1615	0,478		
				3714	0,478		

v		VII	
Strain	Distance	Strain	Distance
1815	0,492	3814	
3514	0,610		
4217	0,668		
1914	0,714		
2314	1,018		

Supplementary Table S3. Phytosanitary products used at Melgarajo

2014	
Agent	Description
TOPAS (TOPAS 100EC)	penconzaole 10%; control of powdery mildew
THIOVIT JET	sulfur 80%; control of powdery mildew, rust, mites
CHOKÉ	
PROFOL PLUS 6-30-37	
AZUFRE ESPOLVOREO OROFLEX	sprayable sulfur; broad spectrum fungicide
ASSISTAN	pre- and post-emergence herbicide
ATILA	glyphosate 36%; post-emergence herbicide
LATINO	miclobutanil 12.5%; control of <i>Odium</i> spp (powdery/downy mildew)
CYCLO PLUS	folpet 40% + metalaxyl 10%; control black rot, mildew
APACHE	chlorothalonil 37.5% + propiconazole 6.25% + cyproconazole 5%; broad spectrum fungicide
SFENVALO STAR	esfenvalerate 2.5%; insecticide
NUTRACEICO	
EMERALD	Fluazinam 50%; fungicide
AMICOS COMBI	Plant extract; anti fungal sporulant, micronutrient supply (Mn, Zn)
SUFREVIT	sulfur 80%; fungicide, acaricide
CERCOBIN	broad spectrum fungicide; polyvalent; systemic and contact.
2015	
Agent	Description
ATILA	glyphosate 36%; post-emergence herbicide
PROWL	pendimethalin 33%; herbicide
MEDELINON FLOW	linuron 45%; systemic herbicide
FLINT	trifloxistrobin 50%; control of <i>Odium</i> spp
ORTHOCIDE	captan 80%; control of mildew
DEEPEST TIRAM	tiram 50%; broad spectrum fungicide
BRANDA METALAXIL	metalaxyl; control of mildew
LAITRI	miclobutanil 12.5%; control of <i>Odium</i> spp
FANTIC-M	benalaxil M 4% + mancozeb 65%; control of mildew
CALDO BORDELES LAINCO	copper 20% (cuprocalcium sulfate); broad spectrum fungicide
2016	
Agent	Description
TACTIC	organosilicone surfactant
BORIGAN	wetting agent
GRANO DE ORO (Azufre)	98.5% sulfur; fungicide
COBRE LAINCO	copper oxychloride 50%; control of mildew

LAINCOIL ACEITE VERANO	summer oil 83%; pesticide
KARDA GLIFOSATO	glyphosate; post-emergence herbicide
PRINALUM	pendimethalin 33%; herbicide
LAINTRI	miclobutanil 12.5%; control of <i>Odium</i> spp
MELODY COMBI	folpet 56,3% + iprovalicarb 9%; control of mildew
FLINT	trifloxistrobin 50%; control of <i>Odium</i> spp
GLITAN	glyphosate; post-emergence herbicide
2017	
Agent	Description
ZARPA	diflufenican 4% + glyphosate 16%; herbicide
GLITAN	glyphosate; post-emergence herbicide
GLIFOSATEC	glyphosate; post-emergence herbicide
ACILIQ NPK	complex fertilizer (inorganic)
SOLUCION NITROGENADA	nitrogen fertilizer
MICROFER CA	Calcium
MICROFER MG	Magnesium
PROTAMINAL PLUS	amino acids
ARMETIL	folpet 40% + metalaxyl 10%; control of mildew
MICLOBUTANIL	miclobutanil 12.5 %; control of <i>Odium</i> spp
VAMECTIN	abamectina 1.8%; acaricide (pesticide)
AZUFRE LIQUIDO 80 PALLARES	sulfur concentrated suspension; control of red spider mite
COLLIS	boscalida 20% + kresoxim-metyl 10%; control of <i>Odium</i> spp
DELTAPLAN	deltametrin 2.5%; control of cluster moth
KARATHANE STAR	meptildinocap 35%; control of <i>Odium</i> spp
NOVAZUFRE 98.5%	sulfur 98.5%, red spider mite
VIVANDO	metrafenona 50%; control of <i>Odium</i> spp
GLIMUR GREEN	glyphosate 36%; post-emergence herbicide

Supplementary Table S4. Chemical data of Negro Saurí grapes and wine for vintages 2014-2017

Grapes (average data for 15 clones)									
Vintage	°Brix	Density	Sugar (g L ⁻¹)	pH	Total acidity (g L ⁻¹)	Malic acid (g L ⁻¹)	Tartaric acid (g L ⁻¹)	Potassium (mg L ⁻¹)	I.P.T.
2014	24.5 ± 0.7	1.1 ± 0.0	244.4 ± 8.6	No data	No data	1.8 ± 0.3	8.0 ± 0.5	1528.7 ± 281.6	12.7 ± 1.6
2015	24.5 ± 1.2	1.1 ± 0.0	244.3 ± 13.8	3.3 ± 0.1	5.72 ± 0.5	2.3 ± 0.6	5.7 ± 0.6	1534.0 ± 143.2	14.7 ± 3.8
2016	24.4 ± 1.5	1.1 ± 0.0	242.8 ± 17.7	3.4 ± 0.1	4.84 ± 0.4	2.0 ± 0.5	4.8 ± 0.4	1594.7 ± 114.7	14.5 ± 6.7
2017	24.6 ± 0.7	1.1 ± 0.0	245.5 ± 8.6	3.5 ± 0.1	5.98 ± 0.5	2.5 ± 0.3	7.5 ± 0.4	1973.3 ± 98.1	4.3 ± 0.8

Wine (from single fermentations)				
Vintage	Alcohol % (v/v)	pH	Total acidity (g L ⁻¹)	Volatile acidity (g L ⁻¹)
2014	14.12	3.48	4.03	0.36
2015	13.5	3.37	3.9	0.34
2016	13.8	3.55	4.1	0.25
2017	14.5	3.71	3.6	0.25

I.P.T. Total Polyphenol Index (Indice de Polyphénols Totaux)

Total acidity (g L⁻¹ in sulphuric acid)

Volatile acidity (g L⁻¹ as acetic acid)