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Genetical, Morphological and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.)

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Abstract: 'Uva Rey' is considered an Andalusian (Spain) ancient autochthonous cultivar with hard white grapes used for the production of wine and raisins and also for raw consumption. Currently, this cultivar is not included in the official register of Spanish grapevine varieties and there is neither a description nor a characterization that could facilitate its insertion in this register. In order to study this genetic resource, a genetic and morphological characterization of 'Uva Rey' has been carried out in comparison with 'Palomino Fino', the main cultivar in Andalusia (Spain). Additionally, grape must physicochemical characterization and grape berry texture profile analyses were performed. Genetically, 'Uva Rey' was synonymous with the cultivar 'De Rey'. 'Uva Rey' grape must physicochemical results showed a lower sugar concentration and a higher malic acid content compared to 'Palomino Fino' must, while the analysis of the grape berry texture profile proved to be more consistent and cohesive. These results can be attributed to the longer phenological cycle presented by 'Uva Rey'. All these facts could lead to consideration of 'Uva Rey' as a cultivar for the production of white wines in warm climate regions.

Keywords: *Vitis vinifera*; autochthonous cultivar; 'Uva Rey'

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most ancient and important fruit crops worldwide [1]. Around 12,500 cultivars have been registered in the *Vitis* International Variety Catalogue [2]. However, based on their DNA profiles, the number of grapevine varieties is estimated at around 5000, many of them closely related [3,4].

Nowadays, 7.4 mHa of the Earth area is covered by grapevines, with Spain being the first country in terms of cultivated land extension. Spanish vineyards cover thousands of hectares and produce approximately 44.4 mHL of wine per year [5]. For that reason, viticulture could be considered as one of the most important socioeconomic sectors in the Spanish agro-industrial network. Grapevine cultivation throughout the country, and the significance over time, have led to a grapevine heritage of great magnitude. Spain's varietal heritage had continuously increased from its origin until the arrival of diseases and pathogens from America (mildews and *Phylloxera*) [6]. According to García de los Salmones [7], the first *Phylloxera* outbreak in Spain was detected in Malaga (Andalusia) in 1876. From that moment on, this pathogen spread throughout the whole country and destroyed more than 1,000,000 ha, which caused serious damages to the Spanish native germplasm [8]. In order to preserve the maximum number of *Vitis vinifera* genetic diversity, a number of germplasm banks were created. 'El Encín', the most important germplasm bank in Spain, was established in 1914 in Alcalá de Henares

(Madrid, Spain) [9]. Later on, the currently germplasm bank known as 'Rancho de la Merced', was created in 1940, with the first collection of grapevines in Jerez de la Frontera (Andalusia, Spain) [10].

From then on, the prospection, collection and conservation of different grapevine cultivars as a genetic resource have been the subject of numerous studies that intend to preserve those cultivars considered as autochthonous [7–11]. For the identification of that genetic material, molecular characterization using Simple Sequence Repeats (SSR) markers [12], ampelographic [4] and physicochemical [13] techniques have been used. Grapevine genotypes are highly heterozygous and the relevance of near-homozygous lines was not considered until recently due to the need to generate high quality reference sequences [14], and has been maintained in cultivated plants through vegetative propagation [15].

Modern wine industries only use a limited number of *Vitis vinifera* cultivars [16]. In Spain, by virtue of the Spanish Royal-Decree-Law (RD) 1338/2018, only those varieties that have been properly registered can be planted [17]. However, there is a current trend towards the production of genuine and characteristic wines [18]. Currently, the changing climate is expected to impose new challenges to varietal selection. Since grapevine varietal suitability is strongly linked to regional environmental conditions, growers are prone to select varieties that are best suited to these changing agroclimatic factors [19].

As a result, autochthonous cultivars, such as 'Uva Rey' would require to be identified and characterized, since they were already used for wine making in the 19th century in southwestern regions in Andalusia [20]. Roxas Clemente [21] included this variety in Tribe III of the First Section and indicated that it was cultivated under different denominations in different districts within Cadiz and Seville provinces in Andalusia. Regarding its grapes, this author described them as very large, round, somewhat golden and with a long cycle. With regards to its winemaking potential, Abela [22] confirmed that this grape variety was able to produce fine wines with plenty of mouth-feel and acidity.

The main objective of this research work is to complete the characterization of the cultivar 'Uva Rey' as currently kept in a specific vineyard located in Andalusia (Spain). For this purpose, the genetic identification, the ampelographic characterization, the grape berry texture profile analysis and the physicochemical characterization of the grape musts have been carried out.

2. Materials and Methods

2.1. Plant Material and Experimental Design

A total of 10 plants of 'Uva Rey' from a vineyard in the town of Chiclana de la Frontera municipal district (Andalusia, Spain) were selected (lat. 36°27′30.6″ N; long. 6°05′46.2″ W; 69 m above sea level). In addition, 'Palomino Fino' was used as a reference cultivar for all the studies, as it is the most widespread variety in the southwest of Andalusia [23]. Both cultivars were 15 years old and had been grown with the same vine spacing (2.30 × 1.15 m) as well as trained according to the 'Vara y Pulgar' (stick and thumb) system. Additional Figures S1a–c and S2a–c show the temperature, humidity, radiation and rainfall during the period from July (veraison) to September (harvest) for 2016 and 2017 respectively. For the genetic characterization of the cultivar, four varieties: 'Cabernet Sauvignon', 'Chardonnay', 'Muscat a Petits Grains Blancs' and 'Pinot Noir' were included as reference to compare their genotype databases and confirm the new cultivar accession identity (Table 1).

The morphological description and the texture profile analysis (TPA) of the berries as well as the grape must characterization were carried out for 'Uva Rey' and 'Palomino Fino' cultivars from the same vineyard and in two consecutive years (2016 and 2017) in order to study the vintage effect on the different cultivars. Both cultivars were grown at the same vineyard and under the same agroclimatic conditions, the cultural practices and were harvested in the same period (first week in September). In order to minimize variability due to grapevine sampling, Santesteban et al. [24] criterion was applied. For this purpose, the trunk cross sectional area (TCSA) of a total of 50 vines were measured at 30 cm

height using a digital Verner calliper Maurer 93,110 (Padova, Italy). Of all the vines measured, 10 were selected and marked as their TCSA value was the closest to the TCSA average ± 10%.

2.2. Microsatellite Analysis

Two young fresh leaves from each accession were collected at the vineyard and kept at –80 °C until analysis. DNA extraction was carried out using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Varietal identification was performed using 22 nuclear microsatellite loci. The first set of 20 microsatellite loci located in the 19 linkage groups of grapevine genome (VMC1B11 (GeneBank, Accession Number BV681754), VMC4F3-1 [25]; VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD28, VVMD32 [26,27]; VVS2 [28]; VV1B01, VVIH54, VVIN16, VVIN73, VVIP31, VVIP 60, VVIQ52, VVIV37, VVIV67 [29]) were analysed as described by Vargas et al. [30], using two multiplex Polimerase Chain Reactions (PCR). An additional set of two microsatellite loci (VrZAG62 and VRZAG79) [31] were analyed following the conditions described in detail by Jiménez-Cantizano et al. [32], in order to complete the list of loci authorized by the International Organisation of Vine and Wine (OIV). PCR amplifications were performed using a 9700 thermocycler and the amplified products were separated by capillary electrophoresis using an automated sequencer ABI Prism 3130 (Applied Biosystems, Foster City, CA, USA). Fluorescent labelled fragments (6-FAM, VIC, PET and NED) were detected and sized using GeneMapper v. 3.7 and fragment lengths were assessed with the help of internal standards GeneScan-500 LIZTM (Applied Biosystems, Foster City, CA, USA). The microsatellite genotypes obtained after the analysis were compared with the genetic profiles provided by Lacombe et al. [33] and the data contained in the microsatellite databases *Vitis* International Variety Catalogue [34], Rancho de la Merced Germplasm Bank genotype database [35] and the *Vitis* Germplasm Bank at Finca el Encín [25,36,37]. The SSR profiles obtained were compared using the microsatellite toolkit v. 9.0 software [38].

2.3. Morphological Characterization

For the morphological analysis, Benito et al. [39] criterion was followed. A total of 10 young shoots, young and mature leaves, flowers, bunches and berries from each accession were analysed using 34 descriptors from the Organisation Internationale de la Vigne et du Vin descriptor list [40]. Each accession from two different vintages was described by five ampelographers and the modal value was selected as the final description.

2.4. Physicochemical Characterization of Grape Berries and Musts

Grapevine berries (n = 50) were evaluated using a texture-meter (Lloyd Material Testing Machine, West Sussex, UK) fitted with a 2 mm cylindrical flat probe at 1 mm/s. The results regarding consistency, firmness, work of penetration (WoP) and cohesiveness were calculated as the average values for 50 berries.

Once harvested, 5 kg of berries of each cultivar (500 g from each vine) were destemmed, grounded and pressed. pH determinations were carried out using a Crisson-2001 digital pH-meter (Loveland, CO, USA). Sugar concentration (°Bé) was determined using a calibrated Dujardin-Salleron hydrometer (Laboratories Dujardin-Salleron, Arcueil Cedex, France). Total acidity (TA) was calculated according to the official methods of analysis [41]. Ripening index (RI) was calculated following the equation proposed by Hidalgo [42]. Yeast assimilable nitrogen (YAN) was determined according to Aerny [43]. Citric, tartaric and malic acids were assessed following the methodology proposed by Sancho-Galán et al [44]. Organic acids concentrations were obtained by ionic chromatography using a Metrohm 930 compact IC Flex ionic chromatographer equipped with a conductimetric detector on a Metrosep Organic Acids column-250/7.8 (Herisau, Switzerland). Organic acids separation was performed using as eluent H_2SO_4 0.4 mM in a 12% acetone solution with an isocratic 0.4 mL/min flow. All the physicochemical measurements were destructive analysis and were conducted in triplicate to ensure statistical significance.

2.5. Statistical Analysis

Means and standard deviations were calculated using Microsoft Office Excel 2016 for Windows 10. Significant differences were evaluated by two-way ANOVA and Bonferroni's multiple range (BSD) test; $p < 0.05$ was considered significant (GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Microsatellite Analysis

The allele profiles obtained for 'Uva Rey' and the five reference cultivars at 22 microsatellite loci are shown in Table 1. The genotype obtained for 'Uva Rey' was compared with the Rancho de la Merced Germplasm Bank genotype database [14,35], the *Vitis* Germplasm Bank at the Finca El Encín [30,36,37] and European databases [33,34]. 'Uva Rey' showed the same genotype as 'Mantuo de Pilas' kept in Rancho de la Merced Germplasm Bank at 22 SSR loci and 'De Rey' at Finca El Encín at 20 SSR loci.

Table 1. Genetic profiles of 'Uva Rey' and reference cultivars at 22 microsatellite loci. Alleles sizes are given in base pairs.

Locus	'Uva Rey'		'Palomino Fino' [a]		'Cabernet Sauvignon' [a]		'Chardonnay' [a]		'Muscat a Petits Grains Blancs' [a]		'Pinot Noir' [a]	
VVIB01	307	307	291	307	291	291	289	295	291	295	289	295
VMC1b11	184	188	184	188	184	184	166	184	184	188	166	172
VMC4F31	184	190	176	206	174	178	174	180	168	206	174	180
VVMD5	224	232	226	238	228	238	232	236	226	324	226	236
VVMD7	244	246	236	246	236	236	236	240	323	246	236	240
VVMD21	243	249	243	249	249	257	249	249	249	265	249	249
VVMD24	209	209	209	209	209	217	209	217	213	217	215	217
VVMD25	238	252	240	240	238	246	238	252	240	246	238	246
VVMD27	180	182	186	194	176	190	182	190	180	194	186	190
VVMD28	246	248	238	250	236	238	220	230	248	270	220	238
VVMD32	270	270	254	256	238	238	238	270	262	270	238	270
VVIH54	166	168	166	166	166	182	164	168	166	166	164	168
VVIN16	151	153	151	151	153	153	151	151	149	149	151	159
VVIN73	264	264	256	264	264	268	264	266	264	264	264	266
VVIP31	176	190	188	190	188	188	180	184	184	188	180	180
VVIP60	318	326	318	322	306	314	318	322	318	318	318	320
VVIQ52	85	89	85	85	83	89	83	89	83	83	89	89
VVS2	131	142	131	144	137	151	135	142	131	131	135	151
VVIV37	161	161	163	167	163	163	153	163	163	165	153	163
VVIV67	372	375	364	366	364	372	364	372	364	375	364	372
VrZAG62	187	193	187	193	187	193	187	195	185	195	187	193
VrZAG79	242	248	250	260	246	246	242	244	250	254	238	244
Variety [b]	'De Rey'											

[a] Reference cultivars. [b] Prime names according to *Vitis* International Variety Catalogue (VIVC).

3.2. Morphological Characterization

Modal values for the ampelographic descriptions of 'Uva Rey' cultivar corresponding to years 2016 and 2017 are shown in Table 2 compared to the reference cultivar 'Palomino Fino'.

Table 2. Ampelographic description of 'Uva Rey' and 'Palomino Fino' cultivars using the International Organisation of Vine and Wine (OIV) descriptors.

Code	Descriptor	'Uva Rey'	'Palomino Fino'
OIV 001	Young shoot: opening of the shoot tip. 1 closed, 3 half open, 5 fully open.	5	5
OIV 003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	3	5
OIV 004	Young shoot: density of prostrate hairs on the shoot tip. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	7	5
OIV 006	Shoot: attitude (before tying). 1 erect, 3 semi-erect, 5 horizontal, 7 semi-drooping, 9 drooping.	3	3
OIV 007	Shoot: colour of the dorsal side of internodes. 1 green, 2 green and red, 3 red.	1	2
OIV 008	Shoot: colour of the ventral side of internodes. 1 green, 2 green and red, 3 red.	1	2
OIV 015-1	Shoot: distribution of anthocyanin coloration on the bud scales. 1 absent, 2 basal, 3 up to 3/4 of bud scale, 4 almost on the whole bud scale.	1	3
OIV 016	Shoot: number of consecutive tendrils. 1 two or less, 2 three or more.	1	1
OIV 051	Young leaf: colour of upper side of blade (4th leaf). 1 green, 2 yellow, 3 bronze, 4 copper-reddish.	3	3
OIV 053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf). 1 none or very low, 3 low, 5, medium, 7 high, 9 very high.	9	5
OIV 065	Mature leaf: size of blade. 1 very small, 3, small, 5 medium, 7 large, 9 very large.	7	7
OIV 067	Mature leaf: shape of blade. 1 cordate, 3 wedge-shaped, 3 pentagonal, 4 circular, 5 kidney-shaped.	3	3
OIV 068	Mature leaf: number of lobes. 1 one, 2 three, 3 five, 4 seven, 5 more than seven.	3	3
OIV 070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade. 1 absent, 2 only at the petiolar point, 3 up to the 1st bifurcation, 4 up to the 2nd bifurcation, 5 beyond the 2nd bifurcation.	1	3
OIV 072	Mature leaf: goffering of blade. 1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong.	7	5
OIV 074	Mature leaf: profile of blade in cross section. 1 flat, 2 V-shaped, 3 involute, 4 revolute, 5 twisted.	5	4
OIV 075	Mature leaf: blistering of upper side of blade. 1 absent or very weak, 2 weak, 3 medium, 4 strong, 9 very strong.	5	3
OIV 076	Mature leaf: shape of teeth. 1 both sides concave, 2 both sides straight, 3 both sides convex, 4 one side concave on side convex, 5 mixture between both sides straight and both sides convex.	2	3
OIV 079	Mature leaf: degree of opening/overlapping of petiole sinus. 1 very wide open, 3 open, 5 closed, 7 overlapped, 9 strongly overlapped.	3	5
OIV 080	Mature leaf: shape of base petiole sinus. 1 U-shaped, 2 brace-shaped, 3 V-shaped.	3	3

Table 2. *Cont.*

Code	Descriptor	'Uva Rey'	'Palomino Fino'
OIV 081-1	Mature leaf: teeth in the petiole sinus. 1 none, 9 present.	1	1
OIV 081-2	Mature leaf: petiole sinus base limited by vein. 1 not limited, 3 on one side, 3 on both sides.	1	1
OIV 083-2	Mature leaf: teeth in the upper lateral sinuses. 1 none, 9 present.	1	1
OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	7	7
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade. 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	9	1
OIV 151	Flower: sexual organs. 1 fully developed stamens and no gynoecium, 2 fully developed stamens and reduced gynoecium, 3 fully developed stamens and fully developed gynoecium, 4 reflexed stamens and fully developed gynoecium.	3	3
OIV 202	Bunch: length (peduncle excluded). 1 very short, 3 short, 5 medium, 7 long, 9 very long.	5	7
OIV 203	Bunch: width. 1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	5
OIV 204	Bunch: density. 1 very loose, 3 loose, 5 medium, 7 dense, 9 very dense.	5	5
OIV 206	Bunch: length of peduncle of primary bunch. 1 very short, 3 short, 5 medium, 7 long, 9 very long.	3	1
OIV 220	Berry: length. 1 very short, 3 short, 5 medium, 7 long, 9 very long.	5	3
OIV 221	Berry: width. 1very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	3
OIV 223	Berry: shape. 1 obloid, 2 globose, 3 broad ellipsoid, 4 narrow ellipsoid, 5 cylindrical, 6 obtuse ovoid, 7 ovoid, 8 obovoid, 9 horn shaped, 10 finger shaped.	7	2
OIV 225	Berry: colour of skin. 1 green yellow, 2 rose, 3 red, 4, grey, 5 dark red violet, 6 blue black.	1	1

A total of 34 descriptors were studied, eight of which correspond to shoots, 17 to leaves, one to inflorescence, four to bunches and four to berries. In regard to the density of prostate hairs between the main veins on lower side of blade (OIV 053), 'Uva Rey' showed very high density while 'Palomino Fino' prostate hair density was medium. Also, the density of erect hairs on the main veins on the lower side of tthe blade (OIV 087) was high for 'Uva Rey' and non-existent or low for 'Palomino Fino' cultivar. Finally, grape berries were green yellow in both cases (OIV 225), but their shapes differed (OIV 223), being ovoid for 'Uva Rey' and globose for 'Palomino Fino'.

3.3. Physicochemical Characterization of Grapes and Musts

'Uva Rey' and 'Palomino Fino' grape must physicochemical characterizations and berry texture profile analyses (TPA) from two vintages (2016 and 2017) are displayed in Table 3.

Table 3. 'Uva Rey' and 'Palomino Fino' grape berry texture profile analysis (TPA) and must characterization.

	2016		2017	
	'Palomino Fino'	**'Uva Rey'**	**'Palomino Fino'**	**'Uva Rey**
Physicochemical Parameters				
pH	3.93 ± 0.01 ^{a}	3.87 ± 0.07 ^{a}	4.02 ± 0.03 ^{a}	3.97 ± 0.02 ^{a}
Total Acidity (g/L TH_2)	3.74 ± 0.05 ^{a}	3.51 ± 0.07 ^{a}	3.15 ± 0.08 ^{b}	3.25 ± 0.21 ^{b}
Sugar (°Bé)	12.85 ± 0.01 ^{a}	8.45 ± 0.02 ^{b}	11.70 ± 0.02 ^{c}	7.40 ± 0.06 ^{d}
Ripening Index (RI)	3.44 ± 0.02 ^{a}	2.41 ± 0.01 ^{b}	3.71 ± 0.02 ^{a}	2.28 ± 0.01 ^{b}
YAN (mg/L)	200.00 ± 2.00 ^{a}	140.00 ± 2.00 ^{b}	161.00 ± 6.00 ^{c}	140.00 ± 3.00 ^{b}
Tartaric Acid (g/L)	3.140 ± 0.050 ^{a}	2.720 ± 0.008 ^{b}	2. 470 ± 0.100 ^{b}	2.600 ± 0.200 ^{b}
Citric Acid (g/L)	0.030 ± 0.005 ^{a}	0.100 ± 0.001 ^{b}	0.030 ± 0.010 ^{a}	0.150 ± 0.002 ^{c}
Malic acid (g/L)	0.420 ± 0.020 ^{a}	0.650 ± 0.003 ^{b}	0.100 ± 0.020 ^{c}	0.600 ± 0.010 ^{d}
TPA				
Consistency (Nmm)	89.58 ± 1.59 ^{a}	138.24 ± 8.47 ^{b}	93.66 ± 2.27 ^{a}	152.42 ± 11.18 ^{c}
Hardness (Nmm)	237.57 ± 4.58 ^{a}	239.20 ± 7.56 ^{a}	237.29 ± 5.18 ^{a}	245.05 ± 12.08 ^{a}
WoP (Nmm)	260.47 ± 12.87 ^{a}	351.35 ± 14.98 ^{b}	280.13 ± 16.70 ^{a}	409.93 ± 23.70 ^{c}
Cohesiveness	0.21 ± 0.02 ^{a}	0.41 ± 0.02 ^{b}	0.23 ± 0.02 ^{a}	0.40 ± 0.03 ^{b}

Different superscript letters mean statistically significant differences between samples at p-adjust < 0.05 obtained by two-way ANOVA and Bonferroni's multiple range (BSD) test. Results are the means ± SD of three repetitions.

The main differences between 'Uva Rey' and 'Palomino Fino' cultivars grape musts were related to the physicochemical parameters sugar (°Bé), YAN (mg/L), malic acid (g/L) and TPA consistency (Nmm) and cohesiveness. The pH values obtained for both cultivars as well as for the two vintages were all similar. However, both cultivars exhibited very similar acidity in both vintages, with slightly higher values in 2017 (ANOVA p-adjust < 0.05). Regarding grape sugar content, it was significantly higher in 'Palomino Fino' grapes than in 'Uva Rey' from the two vintages studied (ANOVA p-adjust < 0.05). Again, greater sugar values (°Bé) as well as total acidity were measured in 2016 grapes from both cultivars (Table 3). Consequently, Ripening Index (RI) values obtained were significantly greater in 'Palomino Fino' than in 'Uva Rey'. However, very different content levels in both cultivars were obtained for YAN, where 'Palomino Fino' showed significantly higher concentrations of YAN than 'Uva Rey' (ANOVA p-adjust < 0.05), which yielded the same content level in the two vintages under study (Table 3).

Regarding organic acids content, it could be observed that tartaric acid represents over 75% of their total acidity. It can be seen that this particular acid content follows the same trend as the total acidity of the grapes. With respect to citric acid concentration, it was significantly lower in 'Palomino Fino' than in 'Uva Rey' cultivar and did not exceed 150 mg/L in either case. However, 'Uva Rey' showed a significantly higher content of malic acid than 'Palomino Fino' in both of the vintages studied (ANOVA p-adjust < 0.05).

With respect to the results obtained from the TPA, 'Uva Rey' obtained higher values for consistency, WoP and cohesiveness than 'Palomino Fino' in both vintages (ANOVA p-adjust < 0.05). However, no differences were observed between cultivars or vintages with regards to grape berry hardness.

4. Discussion

To identify grapevine cultivars, nuclear microsatellite markers are the most widely used tool, as was demonstrated by the European projects GENRES 081 and GrapeGen06. Regardless of the high degree of heterozygosity existing in the grapevine, the genotype with six microsatellite loci (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62 and VrZAG79) is enough to establish the identity of a variety [6], with the exception of the peculiar case of closely related varieties [35] which requires analysis of more loci. For this reason, as a result of the GrapeGen06 project, an international consensus

was established to increase the number of microsatellite loci to 20, located in different binding groups for correct identification. In this study, the analysis was extended to 22 microsatellite loci. It is very important to use the same microsatellite loci in different studies in order to be able to compare genotypes later. The identification of 'Uva Rey' genotype allowed us to confirm the synonyms of this cultivar with both 'De Rey' and 'Mantúo de Pilas', which have already been registered in the *Vitis* International Variety Catalogue (VIVC) at seven loci SSR [34]. The genetic profile for 15 additional loci is presented in this study and the synonymy between 'De Rey' and 'Uva Rey' is confirmed for the first time with the analysis at 22 microsatellite loci. Along with the cultivar genetic identification and, according to the recommendation for the adequate characterisation of *Vitis* genetic material, an ampelographic description was carried out [45]. Such morphological description has been the method previously used by different countries to have a particular cultivar included in the official lists [45]. The phenotype obtained for the cultivar 'Uva Rey' showed some differences with 'Mantuo de Pilas' as described by García de Luján et al. [46]. Some differences were found in OIV 007, OIV 008, OIV 051, OIV 053, OIV 070, OIV 074, OIV 075, OIV 087, OIV 202 and OIV 221 descriptors. It is worth mentioning, the differences in erect hairs density on main veins on lower side of blade in mature leaves (OIV 087). 'Uva Rey' showed a very high density unlike 'Mantuo de Pilas' with a very low one. Similar phenotypic differences have been found between other cultivars such as 'Garnacha' and 'Garnacha Peluda' [47], both considered as somatic variants.

Due to the high temperatures associated to the current global warming, the period during which the minimal temperatures required for the physiological activities of vines is reached is longer than it used to be, and hence, there is an increment in metabolic rates that have an impact on metabolite accumulation [48,49]. In the last 10–30 years, some major changes have been observed in grape development and ripening patterns, such as premature budbreak, flowering and fruit maturity due to agroclimatic changes [50]

The differences between the two cultivars with regards to pH and total acidity can be attributed to climate variations between the two years studied, as such differences can be found in both cultivars (Figures S1 and S2). RI values confirm the above-mentioned differences between cultivars (ANOVA p-adjust < 0.05), with significant differences between both cultivars regardless of the vintage analysed. The variations of these parameters associated to grape ripening processes may be related with each cultivar's phenological stages. 'Uva Rey' is, unlike 'Palomino Fino' a long cycle cultivar [51]. For this reason, grape ripening stages are not reached at the same time.

Organic acids content in each cultivar could be due to their phenological cycle differences [51]. With regard to tartaric acid content, the values remained similar except for 'Palomino Fino' cultivar in the 2016 year. During the grape ripening process, the production of malic acid decreases [52] since this carboxylic acid is also used by the plant at this stage for energy production [53]. In this way, the different malic acid content levels in each cultivar could be explained by their aforementioned asynchronous phenological cycles. Such difference in malic acid content levels could be relevant to prospective winemaking process, where malolactic fermentation (MLF) could result in wines with a greater microbiological stability and sensory complexity [54]. Some authors argue that higher weather temperatures due to global warming may lead to grape musts with a higher pH, which in turn may promote oxidation reactions [50,55]. In this sense, grapevine cultivars with similar characteristics to those presented by 'Mantúo de Pilas' could lead to the production of wines through oxidative ageing.

The YAN values that have been observed in 'Palomino Fino' musts were higher than those observed in 'Uva Rey' for both vintages. Such differences between the two cultivars may be related to the variations observed in their ripening processes, since YAN content increases in grape berries when ripening [56]. In any case, YAN values remained at a sufficient level for a proper alcoholic fermentation (AF) [57]. Yeast assimilable nitrogen (YAN) is a fundamental element for the correct AF of grape musts; since nitrogen is essential for the completion of some yeasts, its presence is compulsory for yeasts to develop in normal conditions during this biological process [58].

According to the TPA, the two vintages of 'Uva Rey' in the study had a higher consistency, hardness, WoP and cohesiveness. It should be noted that cohesiveness depends on the strength of the pulp internal bonds of the grape berries. This parameter is highly related to the OIV 235 descriptor [40], which is employed for the sensory evaluation of grapes during their ripening process. The results obtained from the TPA could be explained by the lack of synchrony between both cultivars phenological cycles. 'Uva Rey' berries, with a longer cycle, were less ripe and therefore presented a greater turgidity at the time of analysis. Such superior berry turgidity plus its higher consistency and WoP could contribute to protect grape berries from dehydration under Andalusian warm weather conditions (SW Spain). When these results are compared to those obtained by Giacosa et al. [59], it can be observed that 'Palomino Fino' presents similar cohesiveness to 'Perle von Csaba' cultivar (Hungarian white vinification grape). Nonetheless, 'Uva Rey' showed a higher degree of similarity with the cultivar 'Sultanina' (a Turkish white table grape). In view of its grape berry TPA, 'Uva Rey' could be considered as a cultivar with a greater resistance than 'Palomino Fino', mainly because of its greater pulp cohesiveness and consistency. These results might be influenced by the phenological cycle differences observed between the two cultivars studied, where the higher values correspond to less ripe berries. In this sense, these phenotypical traits could increase the cultivar's resistance to drought and to high temperatures, which would make it a more appropriate cultivar for warm dry areas and for global warming conditions.

5. Conclusions

Microsatellite analysis confirmed that 'Uva Rey' is a synonym of 'De Rey' cultivar and a somatic variant of 'Mantuo de Pilas'. With respect to the physicochemical grape must characterization, major differences were found in YAN and malic acid concentration. The TPA showed that 'Uva Rey' grape berries are more cohesive and consistent than 'Palomino Fino' ones. In this sense, 'Uva Rey' can be stated as an autochthonous grapevine cultivar with a long phenological cycle. This study recognizes Uva Rey as a somatic variant of 'Mantuo de Pilas' and as such, supports any actions towards its recovery. According to the results obtained from the different analysis that have been completed on 'Uva Rey' grape berries and musts from two consecutive vintages, this autochthonous cultivar should be further studied and included in the Spanish official register to allow is cultivation.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/9/563/s1, Figure S1. (a) Temperature (°C) (T^a_max, T^a_min, T^a_avg), (b) humidity (%) (H_max, H_min, H_avg) and (c) radiation (W/m^2) and rainfall (L/m^2) between July and September 2016. Figure S2. (a) Temperature (°C) (T^a_max, T^a_min, T^a_avg), (b) humidity (%) (H_max, H_min, H_avg) and (c) radiation (W/m^2) and rainfall (L/m^2) between July and September 2017.

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