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Preliminary Study of Somatic Variants of Palomino Fino (*Vitis vinifera* L.) Grown in a Warm Climate Region (Andalusia, Spain)

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Abstract: Vegetative propagation of *Vitis vinifera* cultivars over hundreds of years has led to the accumulation of a large number of somatic variants of the same grapevine variety. These variants are now considered a working tool to cope with changing environmental conditions as a result of, among others, global warming. In this work, three somatic variants of the major grapevine variety of the South West (SW) of Andalusia (Spain), Palomino Fino, have been genetically and morphologically characterized, as well as their grape musts from two different vintages. The genetic analysis at 22 microsatellite loci confirmed the identity of the three somatic variants that presented the same genotype as Palomino Fino, while the morphological study showed differences between the three somatic variants and Palomino Fino, highlighting the somatic variant Palomino Pelusón. Regarding the physicochemical analysis of its musts, differences were also observed between the somatic variants and Palomino Fino. As a result of all of the above, the use of grapes from somatic variants can be a viable and natural alternative for the production of quality wines in warm climate areas. On the other hand, promoting the cultivation of the somatic variants could contribute to preventing the loss of Palomino Fino intraspecific variability.

Keywords: *Vitis vinifera*; Palomino Fino; somatic variants; simple sequence repeat analysis; warm climate

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most widely cultivated fruit crops in the world [1], used mainly for wine and spirit making [2]. This species presents a wide genetic and phenotypic diversity mainly due to the history of vine cultivation [3] and vegetative propagation, which has allowed the conservation of different cultivars for centuries [4]. Grapevine was one of the first fruit species domesticated, and its vegetative propagation has been practiced since ancient times [5]. During many cycles of vegetative propagation, mutations have appeared spontaneously, some of them leading to phenotypic differences giving rise to different somatic variants or clones [6].

The somatic variations have led to grapevine adaptation and to its evolution under changing environmental and cultivation conditions, this being a source of novel traits [7]. This variation became the base of grapevine clonal selection, starting in Germany in the nineteenth century and continuing in some other European countries such as France, Italy and Spain in the second half of the twentieth century [8]. Initially, the basic aim of clonal selection was to get healthy and highly productive plants [8]. However, the aim of obtaining highly productive plants alongside the trend to cultivate only certain varieties has contributed to the disappearance of many local cultivars [9]. Recently, this trend has

started to change, and some wineries, grape growers and consumers have been looking for new local products considering grape quality as a relevant goal to the detriment of yield [10].

Currently, clonal selection has been postulated as one of the working tools to face the adaptation of grapevine varieties to new conditions and environments [11] due to climate change. In the medium to short term, the International Organization of Vine and Wine (OIV) is undertaking the selection and improvement of new varieties for adaptation to climate change [12]. Since 2016, different resolutions of the Genetic Resources and Vine Selection group (GENET) and several research projects have aimed to facilitate the exchange of plant material and germplasm to improve the research and trade in new grapevine varieties. The conservation of this plant material is not a recent development, since the work of prospection, collection and conservation of different vine varieties has been the subject of numerous scientific studies over the years [13–15]. More specifically, in an area with a warm climate such as South West (SW) Andalusia (Spain), the germplasm bank at Rancho de la Merced preserves different somatic variants of the main grapevine variety in Andalusia, Palomino Fino [16].

Palomino Fino is considered an autochthonous grapevine variety [17], and its cultivation in this region has been known since the sixteenth century [18], becoming the eighth most cultivated in Spain between 1990–2012 [19,20]. Actually, this grapevine variety is predominant in the Marco de Jerez for the production of Sherry wines. The long history of Palomino Fino cultivation has led to a high number of clones, which represents an important genetic source. The first clonal selection programme of Palomino Fino was started by Fernández de Bobadilla [21] and continued by García de Luján et al., selecting 28 clones that are currently preserved in the Rancho de la Merced germplasm bank [22]. These clones have been used for new grapevine plantations since the end of the 20th century. Therefore, it is currently very difficult to find new somatic variants or clones that can meet the current needs of wine makers. In addition, in order to carry out behavioural studies of this new plant material, it needs to be preserved in the same plot and growing conditions. These studies are necessary in order to select new plant material better adapted to the changing climate conditions we are facing.

In this sense, the main objective of this work focuses on the characterization of three different somatic variants of Palomino Fino (Palomino Gacho, Palomino de Jerez and Palomino Pelusón) by means of molecular markers, morphological description and physicochemical analysis of grape musts. Their morphological description and grape must analysis could contribute to the detection of traits that could contribute to the production of new white wines in warm climate areas from varieties that could also be better adapted to warm climates.

2. Materials and Methods

2.1. Experimental Design and Grapevine Samples

Three different somatic variants of the grapevine variety Palomino Fino were chosen for the analysis: Palomino Gacho (PG), Palomino Pelusón (PP) and Palomino de Jerez (PJ). Palomino Fino (PF) was employed as the control. All samples were selected from the same vineyard and plot (latitude 36°34′29.7″ N and longitude 5°49′53.5″ W; 150 m above sea level), located in the municipality of San José del Valle (Cádiz, Spain). Vines were 15 years old and were planted with a SW orientation over a limestone soil and with a 2.4 × 1.2 framework vertically trellised, allowing a plant density of 3472 plants per hectare. No irrigation or fertilization treatments were applied during the studied years, and different conventional phytosanitary products were applied to ensure correct grape development. However, during the year 2017, an outbreak of different fungal diseases affected more than 70% of the plots in the Jerez-Xérès-Sherry zone [23], making it impossible to carry out the study during 2017, having to postpone it to 2018.

In order to minimize the intrinsic variability of sampling different vines in the same plot, Santesteban et al.'s [24] criteria were followed. For that reason, 40 vines of each clone's trunk cross sectional area (TCSA) were measured at 30 cm height using a digital Vernier Caliper 93,110 (Maurer,

Padova, Italy). Of all the vines measured, 10 were selected and marked as their TCSA value was the closest to the average $\pm 10\%$.

Additionally, and only for the genetic characterization, four reference varieties (Cabernet Sauvignon (CS), Chardonnay (CH), Muscat a Petits Grains Blancs (MPGB) and Pinot Noir (PN)) were included as reference varieties to compare the genotype obtained in the analysis and those published in databases in order to confirm the identity of the grapevine variety analysed. Those varieties came from a plot previously described in recently published papers [25].

2.2. Genetic Analysis

A total of 22 microsatellite loci were employed to perform the genetic analysis following the methodology established in recently published papers [26]. Young fresh leaves from each somatic variant and from the reference varieties were collected at the vineyard. A DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was employed to extract the DNA. DNA amplifications were carried out using a 9700 thermal cycler (Applied Biosystems Foster City, CA, USA), and the amplified products were separated by capillary electrophoresis using an automated sequencer ABI Prism 3130 (Applied Biosystems Foster City, CA, USA). The four fluorescent labelled fragments (6-FAM, VIC, PET and NED) were detected and measured using GeneMapper v. 3.7 (Applied Biosystems Foster City, CA, USA), and the fragments were assessed using international 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 the databases *Vitis* International Variety Catalogue (VIVC) [27] and the Rancho de la Merced Germplasm Bank database [28].

2.3. Ampelographic Description

A total of 58 descriptors from the International Organization of Vine and Wine (OIV) Descriptor List [29] were evaluated. To this end, five different ampelographers with different knowledge and expertise described a total of 10 shoots, leaves, bunches and berries from each somatic variant following Benito et al.'s [30] criterion during the years 2016 and 2018. The modal value was selected as the final descriptor.

2.4. Grape Musts Physicochemical Characterization

For the physicochemical characterization, the sampling conditions were the same proposed in recently published papers [25]. pH, sugar concentration ($^{\circ}\text{Bé}$), total acidity, tartaric acid, malic acid and yeast assimilable nitrogen (YAN) were determined in the must of the three Palomino somatic variants studied and Palomino Fino. The analyses were performed in triplicate during the years 2016 and 2018 in order to ensure statistical significance. pH was measured using a digital pH-meter CRISON-2001 (Crison, Barcelona, Spain) equipped with a combined electrode with automatic temperature compensation. Sugar concentration was assessed using a calibrated Dujardin–Salleron hydrometer (Laboratories Dujardin–Salleron, Arcueil Cedex, France). Total acidity was determined following the OIV reference method [31]. The Ripening Index was calculated following the equation given by Hidalgo [32]. The concentration of tartaric and malic acid was determined using an ionic exchange chromatograph (Metrohm 930 Compact IC Flex, Herisau, Switzerland) with a conductivity detector on a Metrosep Organic Acids column-250/7.8 (Metrosep, Herisau, Switzerland) following the conditions given by Sancho-Galán et al. [33]. Yeast assimilable nitrogen (YAN) was determined according to the formal method [34].

2.5. Statistical Analysis

Data means and standard deviations were calculated, and significant differences were evaluated by two-way ANOVA and Bonferroni's multiple range (BSD) test with a $p\text{-adjust} < 0.05$ (GraphPad Prism v. 6.01 for Windows, GraphPad Software, San Diego, CA, USA). A hierarchical clustering

analysis (HCA) using Ward's method and the Euclidean square distance was performed using the statistical software SPSS 24.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Genetic Analysis

The allele profiles obtained for the three somatic variants studied, Palomino Fino and the reference varieties at 22 microsatellite loci are shown in Table 1. All Palomino accessions analysed presented the same genotype. It was compared with the published genotype at the Rancho de la Merced Germplasm Bank genotype database [28] and international databases [27].

Table 1. Genetic profiles of different Palomino variants at 22 microsatellite loci. Allele sizes are given in base pairs.

Grapevine Variety Code	PF, PJ, PG, PP		CS		CH		MPGB		PN		
Microsatellite Locus											
VVIB01	291	307	291	291	289	295	291	295	289	295	
VMC1b11	184	188	184	184	166	184	184	188	166	172	
VMC4F31	176	206	174	178	174	180	168	206	174	180	
VVMD5	226	238	228	236	232	236	226	234	226	236	
VVMD7	236	246	236	236	236	240	232	246	236	240	
VVMD21	243	249	249	257	249	249	249	265	249	249	
VVMD24	209	209	209	217	209	219	213	217	215	217	
VVMD25	240	240	238	246	238	252	240	246	238	246	
VVMD27	186	194	176	190	182	190	180	194	186	190	
VVMD28	238	250	236	238	220	230	248	270	220	238	
VVMD32	254	256	238	238	238	270	262	270	238	270	
VVIH54	166	166	166	182	164	168	166	166	164	168	
VVIN16	151	151	153	153	151	151	149	149	151	159	
VVIN73	256	264	264	268	264	266	264	264	264	266	
VVIP31	188	190	190	190	180	184	184	188	180	180	
VVIP60	318	322	306	314	318	322	318	318	318	320	
VVIQ52	85	85	83	89	83	89	83	83	89	89	
VVS2	131	144	137	151	135	142	131	131	135	151	
VVIV37	163	167	163	163	153	163	163	165	153	163	
VVIV67	364	366	364	372	364	372	364	375	364	372	
VrZAG62	187	193	187	193	187	195	185	195	187	193	
VrZAG79	250	260	246	246	242	244	250	254	238	244	

PF: Palomino Fino. PJ: Palomino de Jerez. PG: Palomino Gacho. PP: Palomino Pelusón. CS: Cabernet Sauvignon. CH: Chardonnay. MPGB: Muscat a Petits Grains Blancs. PN: Pinot Noir.

3.2. Ampelographical Description

Table S1 shows the modal values obtained after the morphological description of the three somatic variants compared to the control for the years 2016 and 2018. All the somatic variants and Palomino Fino presented different phenotypes. In order to sum up the information displayed in Table S1, Table 2 and Figure 1 show the number of differences between the analysed Palomino variants. Table 1 shows Palomino Pelusón as the somatic variant with a greater number of differences with respect to the rest of the studied Palomino variants. This is due to the fact that this somatic variant had higher scores in all the hair-related descriptors (OIV 004, OIV 013, OIV 051, OIV 054, OIV 084, OIV 085, OIV 086, OIV 087 and OIV 088). Additionally, Palomino Fino showed differences with the rest of the somatic variants regarding three of the six analysed descriptors concerning the bunch (OIV 202, OIV 203 and OIV 502).

Table 2. Number of different descriptors between the different somatic variants (Palomino de Jerez, Palomino Gacho and Palomino Pelusón) and the control Palomino Fino.

	Palomino Fino	Palomino de Jerez	Palomino Gacho	Palomino Pelusón
Palomino Fino	X			
Palomino de Jerez	10	X		
Palomino Gacho	8	10	X	
Palomino Pelusón	19	17	17	X

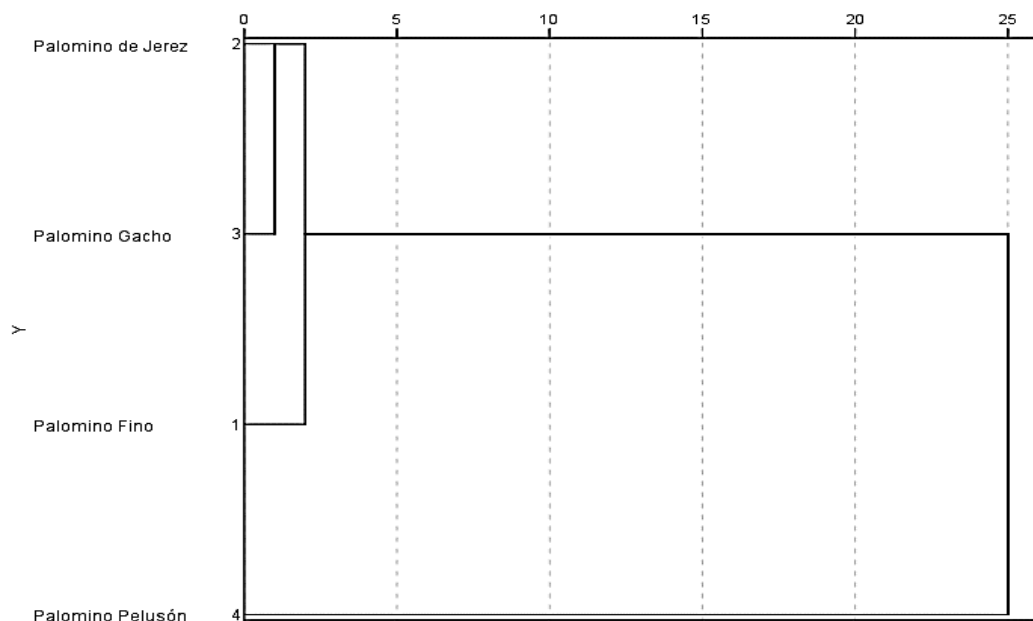


Figure 1. Dendrogram representing the differences among the different variants studied based on ampelographic characterization employing an average link between groups and re-scaled distance cluster combination.

According to the 58 descriptors studied, the HCA analysis (Figure 1) shows how Palomino Pelusón is the most different somatic variant regarding its morphological traits. Furthermore, the rest of the variants analysed are displayed in one group, Palomino Fino being the most different type among this second group.

3.3. Grape Must Physicochemical Characterization

Table 3 shows the result of the grape must physicochemical characterization at harvest from the four Palomino variants analysed during 2016. The pH values ranged from 3.80 for Palomino Fino to 3.53 for the somatic variants Palomino Gacho and Palomino de Jerez. Palomino Fino showed significant differences with the rest of the somatic variants (p -adjust < 0.05). Regarding sugar concentration, the results varied between 12.85°Bé and 10.35°Bé for Palomino Fino and Palomino de Jerez, respectively, with significant differences between all the studied variants. In this way, it can be seen how Palomino Fino was the ripest variant at the time of harvest. In relation to total acidity, this parameter showed values ranging from 4.851 to 3.151 g/L for Palomino Gacho and Palomino Fino, respectively. In this way, it can be seen that Palomino Fino, with the highest sugar content, had the lowest total acidity content, but not the inverse case since the highest values of total acidity were observed for the somatic variant Palomino Gacho and not for Palomino de Jerez. The Ripening Index, calculated from the values of sugar concentration and total acidity, showed the highest value for the Palomino Fino (4.07), while the somatic variant Palomino de Jerez showed the lowest value on the Ripening Index (2.48). The content of the two main organic acids present in the grape must, tartaric acid and malic acid, showed significant differences between all the cultivars studied (p -adjust > 0.05). On the one hand,

tartaric acid content ranged between 4.002 and 2.340 g/L for the Palomino de Jerez and Palomino Fino variants, respectively. However, the malic acid content showed a completely opposite behaviour, showing its maximum content in the control Palomino Fino (0.622 g/L) and its lowest concentration in the somatic variant Palomino Gacho (0.104 g/L). Finally, the YAN content ranged from 247.54 mg/L for Palomino Gacho to 189.27 mg/L for Palomino Pelusón, the values of the former significantly higher than in the other somatic variants studied.

Table 3. Palomino Fino (control) and somatic variants (Palomino Gacho, Pelusón and de Jerez) grape must physicochemical characterization at harvest during the year 2016.

	Palomino Fino			Palomino Gacho			Palomino Pelusón			Palomino de Jerez		
pH	3.87	±	0.01 ^a	3.53	±	0.01 ^b	3.61	±	0.03 ^b	3.53	±	0.03 ^b
Baumé	12.85	±	0.00 ^a	11.98	±	0.09 ^b	11.10	±	0.01 ^c	10.35	±	0.10 ^d
Total Acidity (g/L)	3.15	±	0.05 ^a	4.58	±	0.10 ^b	3.32	±	0.06 ^a	4.17	±	0.06 ^c
Ripening Index	4.07	±	0.02 ^a	2.61	±	0.08 ^b	3.34	±	0.07 ^c	2.48	±	0.01 ^b
Tartaric Acid (g/L)	2.340	±	0.050 ^a	2.460	±	0.062 ^b	2.663	±	0.041 ^c	4.002	±	0.055 ^d
Malic Acid (g/L)	0.622	±	0.064 ^a	0.104	±	0.006 ^b	0.264	±	0.040 ^c	0.200	±	0.009 ^d
YAN (mg/L)	200.16	±	2.13 ^a	247.54	±	2.61 ^b	189.27	±	1.54 ^a	196.47	±	5.69 ^a

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.

Given the significant differences observed in the physicochemical composition of the different grape musts at the time of harvest, it was decided to extend the analysis with different sampling points. During 2018, the control and the three somatic variants were studied during the final ripening stages, sampling every 7 days. Technological ripening parameters such as total acidity and sugar concentration were monitored, as well as tartaric and malic acid concentration given their involvement in some metabolic processes during grape ripening. Figure 2a,b shows the evolution of the above-mentioned parameters during the end of the ripening process until harvest.

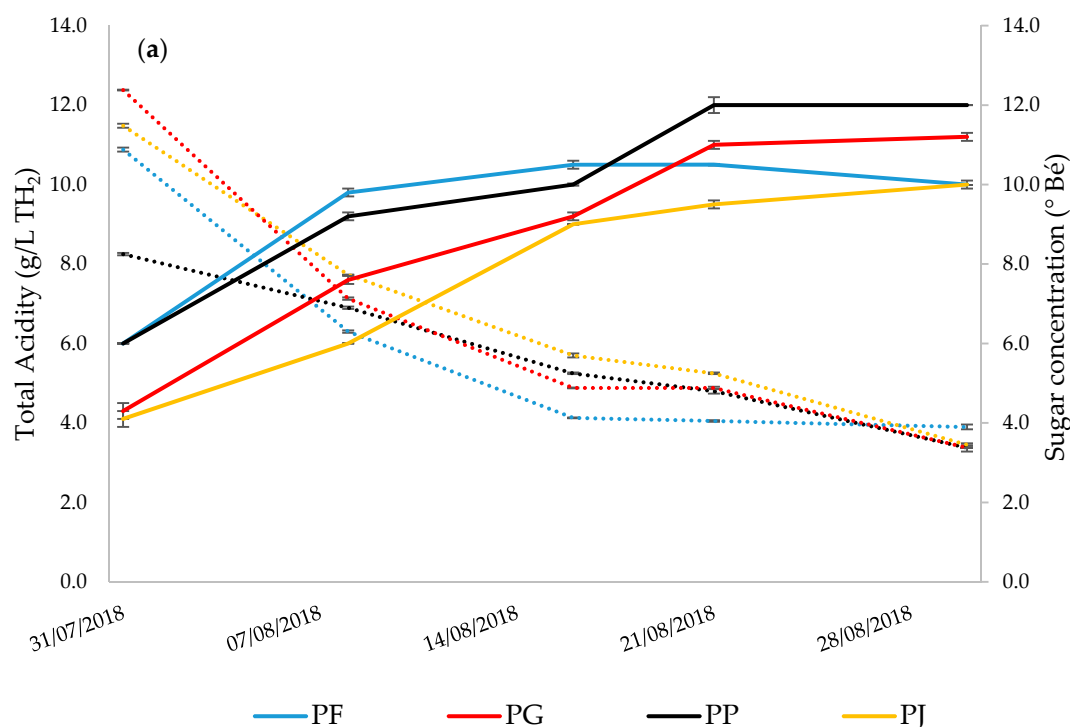


Figure 2. Cont.

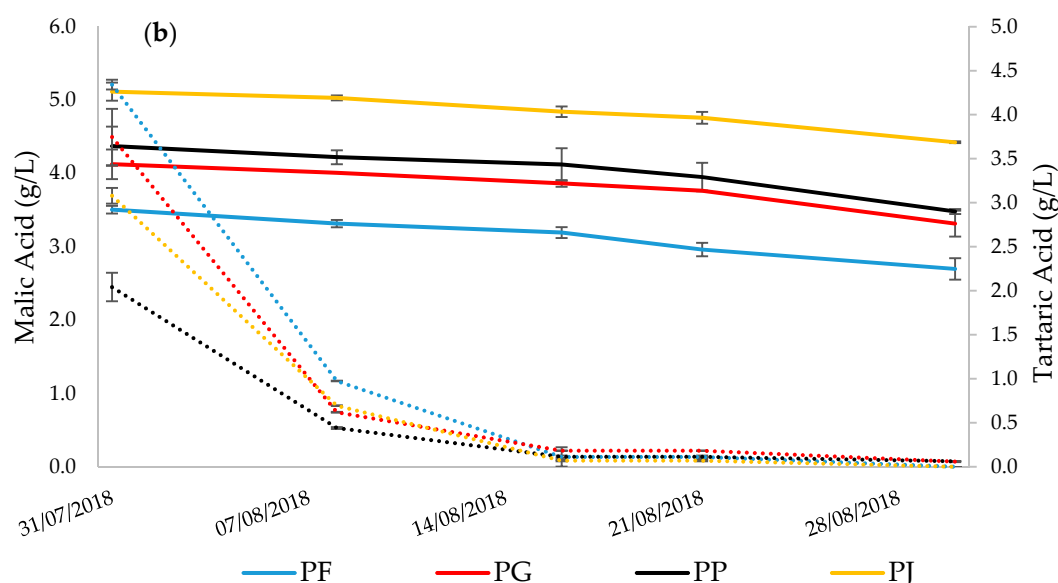


Figure 2. (a,b) Evolution of total acidity (left axis, dotted line) and sugar concentration (right axis, solid line) (a) and malic (left axis, dotted line) and tartaric acid (right axis, solid line) (b) during ripening final stages of Palomino Fino (PF), Palomino Gacho (PG), Palomino Pelusón (PP) and Palomino de Jerez (PJ).

In general, for all the accessions studied, Figure 2a shows a considerable gradual decrease in total acidity during the ripening process. Each cultivar studied starts the ripening process with different acidity values, Palomino Gacho being the somatic variant with the highest total acidity value (12.38 g/L TH₂) and Palomino Pelusón the somatic variant with a significantly lower value (8.25 g/L TH₂) (p -adjust < 0.05). Noting the evolution of this parameter, it can be seen how Palomino Fino undergoes a rapid decrease in total acidity between the 01 August 2018 and 17 August 2018, and then stabilizes until the date of harvest. In the remaining cases, the evolution of the different somatic variants between 17 August 2018 and 31 August 2018 is not so pronounced, with similar final total acidity values and no significant differences between all the Palomino variants studied. With regard to sugar concentration, the most significant increase during the ripening process is observed during the first 16 days of sampling. From this moment on, two different trends can be observed: on the one hand, Palomino Fino and Palomino de Jerez show a similar concentration of sugar until harvest. On the other hand, Palomino Gacho and Palomino Pelusón continue increasing their sugar concentration until 21 August 2018, stabilizing at that time until the harvest date. At the end of the ripening process, sugar concentration of somatic variants and the control ranged from 10° Bé for Palomino Fino and Palomino de Jerez to 12° for Palomino Pelusón.

Figure 2b shows how the tartaric acid content decreases gradually as the grapes ripen. The initial concentration of tartaric acid is different for each somatic variant studied, highlighting the significantly low value of Palomino Fino (3.504 g/L of TH₂) and the significantly high value (p -adjust < 0.05) of the somatic variant Palomino de Jerez (5.114 g/L TH₂). Tartaric acid content shows a linear and constant decrease during the grapes' ripening process, being more pronounced near the end of the ripening. Malic acid content (Figure 2b) shows that both the somatic variants and the control experience a similar decrease of this acid during ripening. In the first phase of the ripening process (until 08 August 2018), there is a sharp drop in the concentration of this analyte, which decreases until 16 August 2018. From then on, the concentration of malic acid remains stable (close to zero) and no significant differences between the different somatic variants and the control can be observed.

4. Discussion

Simple sequence repeat (SSR) markers are one of the most widely used tools in genetic identification of grapevine varieties [35], but it is important to use the same set of microsatellites in every work

to be able to compare the results with those published in different databases. In spite of the high heterozygosity that vine has, a set of six microsatellites is enough to discriminate between two grapevine varieties [36]. However, if the grapevine varieties are highly related, it is compulsory to extend the number of microsatellites. In this study, a set of 22 microsatellite loci comprised of the six proposed by the OIV and the consensus established within the European research projects GENRES 081 and GrapeGen06 were used. In this sense, the analysis of this number of microsatellites allows us to create a unique genetic fingerprint [37]. The use of these 22 microsatellite loci did not allow finding genetic differences between the different somatic variants studied. Similar results in which no genotypic differences were observed at 20 microsatellite loci but morphological differences were observed were obtained by Jimenez-Cantizano et al. [38]. However, clear phenotypical differences were observed over time. This result could be due to mutation or epi-mutation events that take place in single cells that belong to specific grapevine meristem cell layers [38]. In this way, grapevine genetic profile was not affected by somatic variation. However, the genetic profile of Palomino Fino was presented at 22 microsatellite loci, 14 of which are not listed on the *Vitis* International Variety Catalogue (VIVC) [27]. Furthermore, Palomino de Jerez and Palomino Pelusón are listed on the VIVC database as synonyms of Palomino Fino, but Palomino Gacho is not. However, this database does include the accession Listán Gacho as a synonym for Palomino Fino. Jiménez-Cantizano [28] analysed both accessions, preserved in the germplasm bank of Rancho de la Merced (Cádiz, Spain), with 20 SSR loci and presented the same genotype. Phenotypic analysis showed slight differences for the OIV descriptors 202, 204, 206, 502 and 506. Therefore, Palomino Gacho could be considered a synonym for Listán Gacho, and both somatic variants of Palomino Fino.

Despite that vegetative propagation is used in vineyards to multiply plant material and produce descendants identical to the original parent, spontaneous phenotypical variation can occur on some shoots as a result of somatic mutations [39]. In this sense, in order to complete grapevine characterization, and following the recommendations established for an adequate characterization of *Vitis* plant material [40], a complete ampelographic description was carried out. Of the 58 descriptors analysed, 14 corresponded to the primary ones proposed by the OIV to discriminate between varieties [29], and the additional 34 were analysed in order to look for differences between the somatic variants. In these first 14 descriptors' set, differences in 50% were found (OIV 004, OIV 051, OIV 076, OIV 079, OIV 084, OIV 087, OIV 203). These differences in the different organs within the same genotype constitute an interesting genetic resource that could be transferred through classical breeding or genetic engineering in the creation of new cultivars [41]. In this sense, the genetic erosion that the *Vitis vinifera* species is undergoing could be diminished, and the transfer of interesting traits between parents and descendants could also be possible. One of these characteristics of interest could be the one shown by the somatic variant Palomino Pelusón. This somatic variant showed a greater intensity in the expression of those characteristics that imply the presence and density of hairiness (Table S1). High density of erect and/or prostate hairs in any organ can be a trait that could make a grapevine variety better adapted to a warm climate zone. Non-glandular vine hairs or trichomes play a functional role in the plant since they modulate evapotranspiration by restricting air flow between the stomatal pores [42].

Currently, the conditions imposed by global warming are substantially affecting the ripening phase of the grape, as well as other previous processes such as plant bud break and flowering [43]. Thus, in recent years, differences have been observed in the metabolic rates of the vine, and therefore in the production and accumulation of metabolites [44,45]. The high temperatures and consequent high evaporation of water from the plant during the months of fruit ripening make this process difficult [46]. This fact, together with the decrease and irregularity of rainfall, makes the obtention of quality grapes for wine making a difficult task for wine makers. Given this trend, one of the possible solutions to solve the problems being experienced could be the study of the physicochemical composition of musts of different somatic variants. The results observed during 2016 show a general trend between the different somatic variants studied and Palomino Fino. The latter showed a higher maturity at the time of harvest and analysis (higher sugar concentration, lower total acidity value and consequently a

significantly higher maturity index (p -adjust < 0.05)). This fact shows that the control had a lack of synchrony with the somatic variants analysed. Despite this lack of synchrony, the YAN content in all cases was higher than the minimum value required to carry out fermentation [47].

Thus, the analysis of different parameters of interest during ripening has shown that differences between somatic variants and the control were observed during the two years of study, confirming in a preliminary way that the differences are inter-annual and that they are specific to each somatic variant. Regarding sugar concentration in the second phase of the study, the evolution observed between the first and the ninth day could be due to the effect of the high temperatures of those days, exceeding 40 °C (Figure S1). Temperature has a direct influence on sugar content [48]. An increase in temperature leads to increased transpiration and a greater transfer of sugars to the fruit [30]. In addition, in areas with high temperatures and a great amount of sunlight present, photosynthesis is encouraged, increasing CO₂ fixation and its conversion into sugars that are transported to the fruit [49]. Furthermore, grapevine production is also a very influential factor in the ripening process, largely determining the final state of ripeness of the vines [50]. This fact could explain the differences in the evolution of some of the somatic variants studied, since the control Palomino Fino presented the highest production of all of them (497 g/bunch), and the process of accumulation of sugars stopped on the 17th day, while Palomino Pelusón experienced the opposite effect, being the somatic variant with the lowest production of grapes (269 g/bunch) and able to accumulate sugars until the end of the ripening process. In this sense, it is clear that Palomino Fino, unlike the other somatic variants, was selected for its high yield. However, in the case of varieties employed for the production of Sherry wines, it would be advisable to select those that have a longer phenological cycle and mature later, thus allowing wines with a higher alcohol content to be obtained and minimizing the addition of alcohol involved in the production of these kinds of wines.

As far as the evolution of total acidity is concerned, the differences described (Figure 2a) are considered normal, since during ripening, different physical-chemical processes take place that lead to a reduction in the acid fraction of the berries and, therefore, to a decrease in total acidity and an increase in pH [51]. The great decrease in the acid fraction of the must may be due to the high temperatures observed in the first phase of ripening (Figure S1a–c), which produces an increase in the respiratory combustion phenomena of malic acid [52,53]. Theoretically, the best weather conditions for optimum ripening of sherry grapes include sunny but not excessively hot weather [54]. If temperatures exceed 38 °C for 4–6 consecutive days, fruit ripening stops and the musts obtained under these conditions have high pH values and low sugar and acid content [55].

When selecting the appropriate cultivars for the production of wines according to the parameters studied in this section, it is essential to take into account that warm regions such as SW Andalusia (Spain) tend to have high values of sugars and low values of acidity, which is a problem when producing table wines, which are too soft [56]. Therefore, it would be interesting to select those grapevine varieties or somatic variants with greater acidity values in case of early vintages (PG, PP, PJ).

Tartaric and malic acids (Figure 2b) represent 70–90% of the acid fraction of the grapes [57], showing the most important differences when comparing the behaviour of the somatic variants and Palomino Fino [58]. The tartaric acid content of each cultivar is due to differences in adaptation to the environment or possible somatic differences [58]. As in this case all the accessions were planted in the same plot, we can assume that these differences in tartaric content between accessions were determined by somatic differences between them. The decrease in tartaric acid content during ripening is mainly due to its salification and the formation of tartrate salts [59], as well as dilution processes caused by the increase in berry size, accentuating this effect in the moments close to the harvest [53]. The tartaric acid content is hardly influenced by the effect of high temperatures, as its concentration does not change much in the first nine days of the ripening final stages. This is due to the fact that tartaric acid is not a substrate for the respiratory combustion of the grain and remains practically constant during the ripening process [60].

As for the malic acid content, the drastic decrease in malic acid concentration does match the period of high temperatures in the first week of August. This is because the high temperatures favour its combustion in the grape cells. Malic acid combustion occurs during ripening, when the plant switches from using carbohydrates as an energy source to using organic acids (including malic acid) [53]. This, in addition to the decrease in malic acid synthesis during the ripening process, results in a significant decrease in malic acid concentration in the fruit [61]. It is important to take into account the strong influence of temperature on respiratory processes, which increase whenever temperature rises and vice versa [52]. For this reason, in periods when there are significant increases in temperature, lower concentrations of malic acid are obtained in wines, which mean lower total acidity and a higher pH. It should also be noted that the Palomino Fino usually has low levels of malic acid [58]. Therefore, in general terms, it could be said that the decrease in total acidity that occurred in the different accessions was mainly due to the combustion of malic acid in the early stages of maturation and the salification and dilution of tartaric acid in its final stages.

5. Conclusions

Genetic analysis at 22 loci microsatellites confirmed the identity of the three somatic variants that presented the same genotype as Palomino Fino. However, the morphological analysis of the plants did show differences between the different variants studied. The greater presence of hairs in the different organs of Palomino Pelusón may give it a greater adaptation in hot climate areas. After the physicochemical analysis, it was observed that there was a lag in the phenological cycles of the variants studied, the somatic variants having a longer phenological cycle than the control variety Palomino Fino; this fact is beneficial for the production of white wines in early vintages in warm climate areas. As a result of all the above, the use of grapes from somatic variants can be a viable and natural alternative for the production of quality wines in hot climate areas, as well as for preventing the genetic erosion of the *Vitis vinifera* species. In addition, promoting the cultivation of the somatic variants could contribute to preventing the loss of the intraspecific variability of Palomino Fino.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2073-4395/10/5/654/s1>, Figure S1 (a) Humidity (%) (H_max, H_min, H_avg), (b) Temperature (°C) (T_max, T_min, T_avg) and (c) radiation (W/m²) and rainfall (L/m²) among July and September 2018. Table S1. Ampelographic description of Palomino Fino (PF), Palomino de Jerez (PJ), Palomino Gacho (PG) and Palomino Pelusón (PP) using the International Organization of Vine and Wine (OIV) descriptors.

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