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Article

Characterization of New Grapevine Varieties Cross-Bred from Monastrell, Authorized for Winemaking in the Warm Region of Murcia (South-Eastern Spain)

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Abstract: Crossbreeding programs allow the selection of new genotypes with better agronomic and oenological properties for the production of quality wine, and allow the development of a more sustainable form of viticulture. This paper describes the white genotype 'Calblanque', and the red genotypes 'Calnegre', 'Gebas' and 'Myrtia', the first wine grape varieties registered by the Instituto Murciano de Investigación y Desarollo Agrario y Medioambiental (IMIDA) as commercial varieties after confirming the winemaking quality of their grapes in a semi-arid climate with high temperatures. These new varieties have recently been authorized for winemaking in the Region of Murcia. 'Calblanque', 'Calnegre' and 'Gebas' were obtained from crosses between 'Monastrell' and 'Cabernet Sauvignon', and 'Myrtia' from crosses between 'Monastrell' and 'Syrah'. The red genotypes were selected for their phenolic quality—which was very superior to that of the parentals—and for their different harvest dates that allow a staggered harvest and their cultivation in different areas. 'Calblanque' was selected for its good balance of acidity and aromatic profile. The attributes of these new varieties could allow their better adaptation to the effects of climate change on grape and wine quality in warm areas.

Keywords: breeding; quality; distinctness; uniformity; stability; sustainability; wine grape

1. Introduction

Wine quality is associated with physicochemical parameters of the grape such as the accumulation of minerals, sugar, amino acids and organic acids, and the synthesis of flavor and aroma compounds [1,2]. In wine, the acidity is essential for its conservation and good evolution over time, as well as for its organoleptic properties, so a reduction in total acidity can lead to unbalanced and flat wines [3–5]. Particularly in red wines, there is a relationship between wine quality attributes such as aroma, color and body, and the high phenolic content of the berry [6,7]. Nevertheless, high temperatures have been correlated with a reduction in acidity, and with a greater and faster synthesis of sugars and anthocyanins, although at temperatures above 35 °C, anthocyanins stop accumulating and may even be degraded depending on the variety [8–10].

One of the long-term strategies for adapting wine production to hot climates is the selection of suitable plant material (variety/clone and rootstock) from the existing vine biodiversity [11–17]. Another alternative is the selection of crossbreeding better adapted to the specific conditions of the viticulture zone [14,18,19] while still showing good agronomic properties, grape quality and enological characteristics [20,21]. The need to develop a sustainable viticulture model has led to different grapevine improvement programs designed in order to achieve this goal [14,22].

The Instituto Murciano de Investigación y Desarollo Agrario y Medioambiental (IMIDA) in Murcia (Spain) has been running a program to develop grapevine varieties with better phenolic quality for semi-arid wine-producing areas since 1997, which was initiated by Adrián Martínez-Cutillas who was responsible for the Viticulture and Enology Department at IMIDA. The program is based on new genotypes obtained from crosses between 'Monastrell' and other varieties such as 'Cabernet Sauvignon', 'Syrah', 'Tempranillo', 'Verdejo' or 'Barbera' [23,24]. 'Monastrell' is cultivated in Spain (particularly in the southeast), in France (where it is known as 'Mourvedre'), California (where it is known as 'Mataró'), Chile and in Australia (https://www.oiv.int (accessed on 25 April 2022)). In Spain it is the main variety grown in semi-arid Mediterranean climate zones such as the Jumilla, Bullas and Yecla Denominations of Origin (occupying 81% of the cultivated area). The first outcomes of these classical type of crossbreeding were the 'Calblanque', 'Calnegre', 'Gebas' and 'Myrtia' varieties. These four varieties were added to the list of commercial varieties, both at national and community level, on 25 March 2022 (https://www.boe.es (accessed on 25 March 2022)), via the Spanish Plant Variety Office (OEVV), entrusted by the CPVO to carry out DUS (distinctness, uniformity and stability) tests of vine varieties (*Vitis vinifera* L.) and vine rootstocks. The ampelographic characteristics of the new varieties are available on the website of the Ministry of Agriculture, Fisheries and Food (https://www.mapa.gob.es/ (accessed on 28 March 2022)), in the National and Community Catalogs of the Spanish Office of Vegetable Varieties. These characteristics were monitored for four years (2018–2021) using the CPVO technical protocol (CPVO-TP/050/2) based on the UPOV guidelines and descriptors (TG/50/9) (UPOV 2008; https://www.upov.int/edocs/tgdocs/en/tg050.pdf (accessed on 1 March 2017)). Recently (on 1 March 2023), these varieties were added to the list of varieties that could be grown in the wine-growing area of Murcia for winemaking (https://www.boe.es (accessed on 1 March 2023)). Therefore, the time that has taken from the starting of the breeding program (1997) to the acceptance of the use of these new varieties for the production of wine in the Region of Murcia (2022) has been 26 years. In addition, certified material of these varieties is available for exploitation and multiplication by nurseries, via the Vine Health Certification service of the IMIDA entrusted by the Spanish Ministry of Agriculture, Fisheries and Food to carry out the corresponding tests to evaluate the health status of the vine.

For all of the above, the main objective of this breeding program was to develop varieties with better winemaking quality of their grapes that may be suitable for cultivation in warm Mediterranean climate conditions. We hypothesized that some offspring from the crossings of these parental lines could inherit better agronomic and oenological properties for the production of quality wine in our climate conditions. The attributes of these new varieties could allow their better adaptation to the effects of climate change in semi-arid areas and the development of sustainable viticulture.

2. Materials and Methods

2.1. Study Site and Origin of the Genotypes

Supplementary Tables S1 and S2 show the average distribution of some meteorological parameters recorded in the experimental farm 'Hacienda Nueva' (38°06′40.7″ N; 1°40′50.3″ W; altitude 433 m) where the crossbreeding was carried out.

The plant material include four new genotypes: three selected from crosses between 'Monastrell' (M) and 'Cabernet Sauvignon' (C)—'Calblanque' (MC180), 'Calnegre' (MC80) and 'Gebas' (MC98)—and one between 'Monastrell' (M) and 'Syrah' (S)—'Myrtia' (MS10). All genotypes were unequivocally identified by the analysis of eight simple sequence repeat (SSR) markers via PCR [25] as shown in Table 1, confirming the parental varieties: 'Monastrell' and 'Cabernet Sauvignon' for 'Calblanque', 'Calnegre' and 'Gebas', and 'Monastrell' and 'Syrah' for 'Myrtia'.

Table 1. Genetic profile of the parental varieties and the four new varieties analysed with eight microsatellite loci.

Variety	VMC1A12		VMC8G6		VVMD27		VVMD5		VMC1E11		VMC5E9		VVMD28		VVIV67	
Monastrell	119	137	139	173	177	187	223	238	188	194	214	228	243	256	357	364
Cabernet S.	121	150	161	165	173	187	229	238	192	196	195	218	233	235	364	372
Syrah	137	150	169	173	187	189	223	229	196	206	218	222	217	227	361	381
Calblanque	121	137	165	173	187	187	223	229	188	192	195	228	233	243	357	372
Calnegre	121	137	165	173	187	187	223	238	194	196	195	214	233	256	357	364
Gebas	119	121	139	165	173	177	223	238	188	196	214	218	235	243	357	372
Myrtia	137	150	139	169	187	189	223	229	188	206	214	218	217	256	357	381

Alleles expressed in base pairs (bp).

2.2. Experiment Set Up

In a first phase, evaluation of a total of 1591 offspring from the crossings began when the vines were three years old (one plant per genotype from the germination of a seed), at the same locations as the crossing. This phase of selection was based on the quality of the grape and on the adequate agronomic behavior of the plant. In a second phase, twenty-five plants per genotype preselected in the first phase were grafted onto 110-Ritcher rootstocks for a more comprehensive study in which the quality of the wine was also included. 'Calnegre' was grafted in 2003, before Calblanque' (2007), 'Gebas' (2012) and 'Myrtia' (2012). The cultivation techniques—training system, fertilizer use, phytosanitary treatments and soil maintenance—were the same throughout the experimental plot.

The selection criteria of the genotypes for their registration as commercial varieties were based on different dates of ripening, in order to allow a staggered harvest, and in the quality of the grape and wine, using 'Monastrell' as the reference cultivar in the area. Concerning the grape quality, in the case of red grapes were selected genotypes with pH values $\leq$ 3.8, content in anthocyanins > 2000 mg kg^{-1} berry and total phenols > 2700 mg kg^{-1} berry. In the case of white grapes genotypes were selected with pH values $\leq$ 3.5 and content of malic acid > 2.0 g/L. About the wine quality, the parameters used were the total polyphenol index (TPI) and color intensity (CI), looking for crosses with more than 80 TPI and more than 40 CI.

In 2017, ten scions per selected genotype were grafted onto 110-Ritcher rootstocks. In 2018, the grafted genotypes were sent to the OEVV qualified technical testing center for conducting DUS examinations for four years (2018–2021). Previously, it was verified by serological methods (DAS-ELISA test) that these plants were free of viruses [26], such as three grapevine leafroll-associated viruses (GLRaV-1, 2, 3), grapevine fleck virus (GFkV), grapevine fanleaf virus (GFLV) and arabis mosaic virus (ArMV). The serological test was carried out by the Vine Health Certification service of the IMIDA. In March 2022 the varieties were added to the list of commercial varieties, and certified as virus-free material thanks to the collaboration of the Spanish Ministry of Agriculture, Fisheries and Food with the IMIDA Vine Health Certification service. Finally, in March 2023 they were added to the list of varieties that could be grown in the wine-growing area Murcia for winemaking (BOE of 1 March 2023).

2.3. Sampling and Measurements in Grapes

The plant material was characterized in triplicate (four plants per replica) over 5 years (2017–2021) by the phenological, agronomic and quality level of grapes and wines. The dates for the different phenological stages—budbreak, flowering, veraison and harvest—for each genotype were recorded [27]. The date of budbreak was considered when vines reached BBCH stage 09 (green shoot tips clearly visible); the date of flowering when vines reached BBCH stage 65 (50% of flowerhoods fallen); the veraison date when vines reached BBCH stage 85 (softening of berries); and the date of harvest when vines reached BBCH stage 89 (physiological maturity). Physiological maturity was deemed to begin when the grape reached its maximum size and its highest concentration of sugars. At this point, the berry begins to decrease in size due to water loss and some dehydrated berries appear in the cluster, the organoleptic maturity of the skin is good, and the seeds are mature (brown color).

For each genotype, total yield (kg/vine) and the weight of 100 randomly selected berries were assessed at harvest time.

The grape quality was assessed at the IMIDA experimental winery. For each replicate, 350 berries were randomly selected from the different areas of the bunches. From this representative sample, 30 berries were taken for the extraction and analysis in triplicate of the total phenolic content (TPC) (mg/kg berry), and of the total anthocyanins (TA) (mg/kg berry) [28]. The rest of the berry sample (320 berries) was crushed, without breaking the seed, and centrifuged. The °Brix value (OIV-MA-AS2-02), total acidity (OIV-MA-AS313-01), must pH (OIV-MA-AS313-15), tartaric acid content [29] and malic acid content (OIV-MA-AS313-11) were analyzed in the must obtained by centrifugation [24].

2.4. Winemaking

Grapes were transported to the winery located in Jumilla (Murcia, Spain), where wines were elaborated in accordance with a traditional vinification protocol in 100 L steel tanks. For red wines, grapes were destemmed, crushed and sulphited (50 mg SO_2/kg). Commercial yeast (Zymaflore FX10 *Saccharomyces cerevisiae*) (Laffort, Bordeaux, France) was used in a dosage of 20 g/100 kg. During alcoholic fermentation (conducted with a temperature adjustment of 25 °C) a daily punching of the tank was made. At the end of alcoholic fermentation, two rackings were carried out, and then pomace was pressed at 1.5 bars in a 75 L tank membrane press. For white wines, the sulphite was added in destemming, crushing, pressing and settling tank. Defanging was static using cold and pectolytic enzymes and then acidity correction was made. There was no strict control of the fermentation temperature, as varietal aromas were sought. Once the alcoholic fermentation was finished, it was racked, sulphited and kept cold. Samples were analyzed in triplicate at the end of alcoholic fermentation.

2.5. Measurements in Wines

The wine characteristics were assessed using different physicochemical parameters following the methodology described by the OIV: alcohol content (OIV-MA-AS312-01), total acidity (OIV-MA-AS313-01), pH (OIV-MA-AS313-15), relative density 20/20 (OIV-MA-AS2-01), total dry extract (OIV-MA-AS2-03B), color intensity and taint (OIV-MA-AS2-07B).

Regarding spectrophotometric parameters, color was measured using the CIELab space, using illuminant D65 and 10° standard observer conditions. The parameters measured were: L* (lightness), a* (from green to red), b* (from blue to yellow) and C* (chroma or saturation) (OIV-MA-AS2-11). These parameters were measured with the spectrophotometer Shimadzu UV-180 (Shimadzu Corporation, Kyoto, Japan). Total polyphenol index (TPI) was analyzed measuring the absorbance at 280 nm [30]. Total anthocyanins (TA) by the method proposed by Ho et al. (2001) [31]. All these parameters were analyzed using the autoanalyzer Miura One (TDI, Barcelona, Spain).

Organoleptic evaluation was carried out using the OIV score sheet for still wines defined in annex 3.1 of Resolution OIV/Concours 332A/2009 (https://www.oiv.int/public/medias/1852/oiv-concours-332a-2009-es-signe.pdf (accessed on 1 March 2017)), and the tasting panel was formed by staff of the Oenological Station previously trained.

2.6. Statistical Analysis

The collected data were subjected to analysis of variance (ANOVA), using StatGraphics Centurion XVI v.16.1.18 software (StatGraphics Technologies, Inc., The Plains, VA, USA). Means were compared according to the LSD test ($p < 0.05$).

3. Results and Discussion

3.1. Ampelographic Characteristics

Some of the ampelographic characteristic are presented in Figure 1 and Supplementary Table S3, which indicates its UPOV descriptors and the note assigned in parentheses.

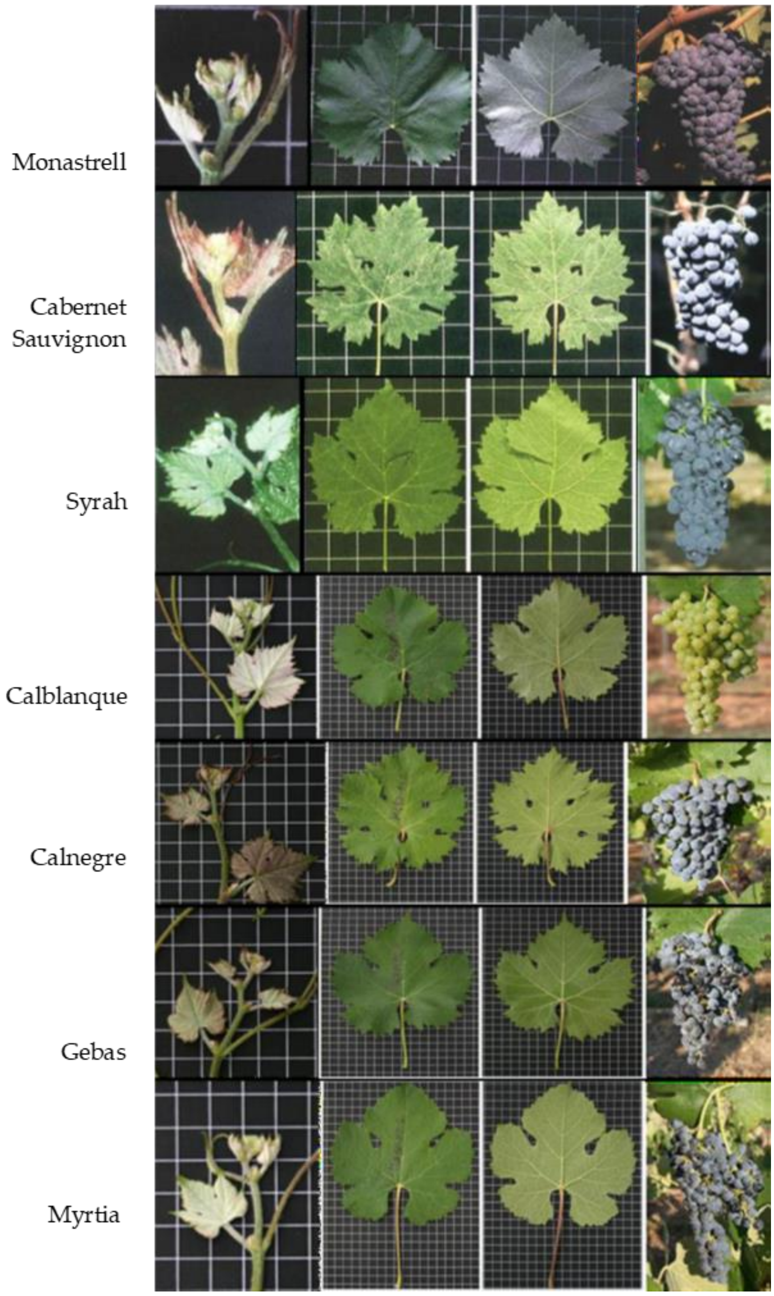

Figure 1. The young shoot, upper and lower side of the mature leaf, and bunch of 'Monastrell', 'Cabernet Sauvignon', 'Syrah', 'Calblanque', 'Calnegre', 'Gebas' and 'Myrtia'. Images obtained from the website of the Spanish Ministry of Agriculture, Fisheries and Food (https://www.mapa.gob.es/ (accessed on 28 March 2022)).

The tip of the young shoot is fully open in all new varieties, and the flowers have fully developed stamens and fully developed gynoecium, except 'Myrtia', which has reflexed stamens and fully developed gynoecium. The mature leaf is circular with seven lobes in 'Calnegre', pentagonal with five lobes in 'Calblanque' and 'Myrtia,' and pentagonal with three lobes in 'Gebas'. The proportion of the main veins on the upper side of the blade with anthocyanin coloration is absent or very low in 'Calblanque', 'Gebas' and 'Myrtia', and medium in 'Calnegre'. The postrate hairs between the main veins on the lower side of the blade have a medium density in 'Calblanque' and 'Myrtia', and a sparse density in 'Calnegre' and 'Gebas'. The bunch is a medium size and has lax to medium density in 'Calblanque' and 'Gebas', a medium size and lax density in 'Myrtia', and a small size and medium density in 'Calnegre'. The berries have a small size, globose shape, blue black color of skin and a particular, different flavor of muscat, foxy or herbaceous in 'Calnegre', 'Gebas' and 'Myrtia', and a small to medium size, globose shape, yellow green color of skin and no particular flavor in 'Calblanque'. The main color of the woody shoot is yellowy brown in 'Calblanque' and 'Calnegre', orange and brown in 'Gebas', and dark brown in 'Myrtia' (Supplementary Table S3).

The main differences according to the UPOV descriptors between the parentals 'Monastrell' and 'Cabernet Sauvignon' and the new variety 'Calblanque' are in the number of lobes and teeth shape of the mature leaf, bunch size and density, and the color of the skin. In the case of the new varieties 'Calnegre' and 'Gebas', the main differences are in the postrate hairs between the main veins on the lower side of the blade, bunch density and the particular flavor of the berries. Finally, the main differences between the parentals 'Monastrell' and 'Syrah' and the new variety 'Myrtia' are in the sexual organs of the flower, the length and teeth shape of the mature leaf, bunch density, and the particular flavor of the berries (Supplementary Table S3).

The complete ampelographic information is available on the website of the Spanish Ministry of Agriculture, Fisheries and Food (https://www.mapa.gob.es/ (accessed on 28 March 2022)), in the National and Community Catalogs of the Spanish Office of Vegetable Varieties (https://www.mapa.gob.es/app/regVar/BusRegVar.aspx?id=es (accessed on 28 March 2022)).

3.2. Phenological, Agronomic and Qualitative Characteristics

In our experimental conditions, 'Syrah' was the earlier parent for all the phenology-related traits, while 'Monastrell' was the latest parent (Table 2), harvesting 'Syrah' 35 days before 'Monastrell'.

Table 2. Mean data (2017–2021) for the phenological stage dates of parental varieties and the new varieties.

Variety	Budbreak	Flowering	Veraison	Harvest	Harvest Days before Monastrell
Monastrell	22 April cd	02 June	10 August c	27 September c	0 a
Cabernet S.	16 April bc	27 May	05 August bc	09 September b	18 c
Syrah	08 April a	23 May	22 July a	23 August a	35 e
Calblanque	13 April ab	24 May	08 August c	25 August a	33 d
Calnegre	21 April cd	30 May	12 August c	11 September b	16 b
Gebas	24 April d	31 May	09 August c	09 September b	18 c
Myrtia	10 April ab	24 May	29 July ab	23 August a	35 e

Different letters in the same column indicate significant differences among genotypes at the 5% level, according to the LSD's multiple range test.

The length of the growing season (from budbreak to harvest) ranged from the 137 days of 'Syrah' to the 158 days of 'Monastrell'. Taking into account the mean harvest date, none of the new varieties were harvested later than 'Monastrell'. 'Calblanque' (white new variety) was harvested 33 days before 'Monastrell' and had a length of growing season of 134 days.

With respect to the new red varieties, 'Myrtia' was the earliest for all the phenology-related traits, harvesting 35 days before 'Monastrell' (similar to 'Syrah'), and presenting the shortest length of the growing season (135 days). 'Calnegre' and 'Gebas' were harvested 16 and 18 days before Monastrell, respectively, and had a length of growing season of 143 and 138 days, respectively.

The new varieties obtained have different optimal maturation dates, which will allow a staggered harvest in the winery. On the other hand, the variability found in the phenology of these new varieties will allow their adaptation to different growing areas, depending on climatic conditions.

Regarding productivity parameters of the new varieties (Table 3), the white variety 'Calblanque' was the most productive with values of yield similar to 'Monastrell' and 'Cabernet Sauvignon' (its parentals). 'Calnegre' (red variety) was the least productive with values lower than its parentals. The higher productivity of 'Calblanque' and the lower productivity of 'Calnegre' coincided with a higher and lower weight of its berries, respectively. Nevertheless, previous studies of the 'Calnegre' variety, comparing its behavior under controlled deficit irrigation and under rainfed conditions, showed that 'Calnegre' (MC80) is one of the varieties, among those studied, whose production is least reduced under rainfed conditions [21].

Since the climatic and growing conditions are the same for all varieties, the variation in berry weight could be due to differences in cell number and/or cell volume, which are determined by cell division and cell expansion, respectively [32]. This hypothesis could be verified with new experiments.

Quality must parameters were also analyzed during the five years of characterization of these new varieties (Table 3). Anthocyanins are a type of polyphenol from the flavonoid group that is the red pigment found in grape skins and sometimes in the flesh. Nevertheless, the amount and composition of anthocyanins present in them varies greatly depending on the species, variety, maturity, vintage, region of cultivation and many other factors [33]. Our parentals obtained anthocyanin values ranging between 1000 and 1800 mg/kg berries; however, in our new red varieties, we obtained anthocyanin values ranging between almost 3000 and a little more than 3500 mg/kg berries, thus tripling the value obtained in the 'Monastrell' variety or doubling the values of 'Cabernet Sauvignon' or 'Syrah'. The highest anthocyanin content was obtained by 'Myrtia' with an average value of 3533 mg/kg berries, the other two varieties 'Gebas' and 'Calnegre' showed very similar average values of around 3000 mg/kg berries. As can be observed, our new varieties greatly exceeded the values obtained by their parentals and this fact is called transgressive segregation; it means that we are going to find a large number of crossbreeds in which the anthocyanin concentration is not within the range of concentration of their parental phenotypes, which is frequent in intraspecific crosses and in domesticated populations [34].

The same situation could be observed when the TPC (total phenolic compounds) were analyzed. Among parentals, the highest concentrations were observed in 'Syrah' grapes (2114 mg/kg berries) and the lower quantities in 'Monastrell' grapes (1554 mg/kg berries). 'Cabernet Sauvignon' showed intermediate values between both varieties (1905 mg/kg berries). However, the new red varieties again showed values much higher than those obtained by their parents, highlighting among them the 'Calnegre' variety, which was the one that obtained the highest amount of total polyphenols, followed by 'Myrtia' and finally 'Gebas'.

As can be observed, no large statistical differences were found between parentals and new red varieties with respect to °Brix (Table 3). 'Calblanque' showed the lowest °Brix value probably because it is a white variety and this type of variety is usually harvested with less sugar quantity. One of the strategies to alleviate the effect of high temperatures on the increase in sugar content and, therefore, on the increase in alcoholic strength, is the use of late-ripening varieties that avoid plants suffering high temperatures during the ripening period. Nevertheless, our results show that in our climatic conditions, varieties that are harvested even 35 days before 'Monastrell', such as 'Myrtia', reach their optimum maturity with the same sugar content as 'Monastrell' (Table 3).

Table 3. Mean data (2017–2021) of production and grape quality variables of parental varieties at harvest.

Variety	kg per Vine	kg per ha	Weight of 100 Berries	Anthocyanins (mg kg^{-1} Berry)	TPC (mg kg^{-1} Berry)	° Brix	pH	TA (g L^{-1} Tartaric Acid)	Tartaric Acid (g L^{-1})	Malic Acid (g L^{-1})
Monastrell	3.25 ± 0.67 ab	8648 ± 1400 abc	152.2 ± 11.9 c	1061 ± 86 a	1554 ± 139 a	24.1 ± 0.6 bc	3.95 ± 0.30 b	2.88 ± 0.35 a	4.20 ± 0.19 a	1.33 ± 0.11 a
Cabernet S.	3.62 ± 0.67 b	9658 ± 1797 c	107.3 ± 7.7 a	1287 ± 127 a	1905 ± 81 ab	24.3 ± 0.4 bc	3.94 ± 0.20 b	3.25 ± 0.22 a	4.91 ± 0.17 b	1.9 ± 0.13 b
Syrah	3.53 ± 0.50 b	9427 ± 1338 c	125.5 ± 7.8 ab	1791 ± 148 b	2114 ± 172 b	24.7 ± 0.4 c	3.94 ± 0.19 b	3.29 ± 0.48 a	4.51 ± 0.12 ab	2.35 ± 0.08 c
Calblanque	3.37 ± 0.60 ab	9329 ± 1516 bc	135.8 ± 10.4 bc			20.2 ± 0.7 a	3.54 ± 0.04 a	4.81 ± 0.26 b	4.88 ± 0.26 b	2.91 ± 0.10 d
Calnegre	2.05 ± 0.16 a	5401 ± 1506 a	106.3 ± 4.4 a	2925 ± 93 c	3697 ± 69 d	22.9 ± 0.5 b	3.67 ± 0.06 a	3.51 ± 0.18 a	4.81 ± 0.18 b	1.13 ± 0.15 a
Gebas	2.48 ± 0.29 ab	7262 ± 971 abc	121.7 ± 7.2 ab	2934 ± 160 c	3151 ± 213 c	23.7 ± 0.8 bc	3.97 ± 0.09 b	3.10 ± 0.22 a	4.06 ± 0.22 a	2.20 ± 0.06 bc
Myrtia	2.18 ± 0.28 a	5819 ± 739 ab	107.2 ± 5.2 a	3533 ± 241 d	3521 ± 134 cd	24.1 ± 0.4 bc	3.64 ± 0.07 a	4.32 ± 0.35 b	4.90 ± 0.22 b	2.27 ± 0.13 c

Data expressed as mean value ± standard deviation. TPC, total phenolic content; TA, total acidity. Different letters in the same column indicate significant differences among genotypes at the 5% level, according to the LSD's multiple range test.

With respect to other parameters, pH, total acidity and organic acid were also measured at harvest. In recent years, in warm areas such as ours, a pH increase has been observed with respect to the values normally detected some decades ago. With respect to the results found in our parentals and new varieties, we observed that 'Monastrell', 'Cabernet Sauvignon', 'Syrah' and 'Gebas' showed the highest pH values close to 4, in contrast to 'Calblanque', 'Calnegre' and 'Myrtia' that showed the lowest pH values close to 3.6 (Table 3). Regarding total acidity, the highest mean value was found in 'Calblanque', the new white variety, with a much higher value than the parent varieties. The red varieties also showed acidity total values higher than 'Monastrell', the reference variety of the area, standing out among them 'Myrtia' with a value of 4.32 despite being harvested in August when in our area we reached temperatures close to 40 °C. These results are in agreement with those obtained by other authors who previously reported that organic acid concentration and the relative proportions of malate and tartrate varied according to the genotype at the ripe stage [21,35–37].

During ripening, tartaric acid concentration decreases by dilution due to fruit enlargement, while malic acid concentration decreases through both dilution and respiration [38–40]. Our results showed how 'Monastrell' together with 'Gebas' obtained the lowest values of tartaric acid, followed by 'Syrah'. The rest of the studied varieties showed similar content for this organic acid. Finally, the highest values of malic acid were obtained in the new white variety (2.91) followed by 'Syrah' and 'Myrtia', then 'Gebas', 'Cabernet Sauvignon', 'Monastrell' and finally by 'Calnegre'. It is remarkable that the malic acid content was one of the criteria used for the selection of white varieties in our genetic breeding program, searching genotypes with values greater than 2.0 g/L.

3.3. Wine Characteristics

Wine quality is determined by several factors such as the type (or blend) of grape varieties, the terroir, the viticultural practices, the winemaking techniques, and the aging conditions [41–43]. The variety of grapes is a key factor in determining the wine flavor, especially during the production of premium wines. Different physicochemical parameters were analyzed at the end of alcoholic fermentation (Table 4): alcohol content, total acidity, pH, density and total dry extract.

Table 4. Mean physical–chemical data (2017–2021) of the wines at the end of alcoholic fermentation.

Variety	Alcohol (V/V)	TA (g L^{-1} Tartaric)	pH	Relative Density 20/20	Total Dry Extract
Monastrell	13.89 ± 0.41 bc	7.37 ± 0.22 bc	3.41 ± 0.03 a	0.9922 ± 0.0004 a	27.30 ± 0.13 b
Cabernet S.	13.79 ± 0.46 bc	7.12 ± 0.53 abc	3.47 ± 0.03 ab	0.9928 ± 0.0006 a	27.52 ± 2.17 b
Syrah	14.35 ± 0.46 c	6.06 ± 0.46 a	3.59 ± 0.06 bc	0.9923 ± 0.0003 a	28.13 ± 0.71 b
Calblanque	12.09 ± 0.41 a	6.40 ± 0.40 ab	3.37 ± 0.04 a	0.9919 ± 0.0006 a	20.37 ± 0.86 a
Calnegre	12.94 ± 0.41 ab	7.39 ± 0.31 bc	3.42 ± 0.03 a	0.9957 ± 0.0002 b	32.61 ± 0.90 c
Gebas	13.56 ± 0.41 bc	7.25 ± 0.48 bc	3.64 ± 0.04 c	0.9951 ± 0.0004 b	32.21 ± 1.05 c
Myrtia	13.40 ± 0.41 bc	7.89 ± 0.32 c	3.41 ± 0.04 a	0.9948 ± 0.0004 b	31.80 ± 0.88 c

Data expressed as mean value ± standard deviation. TA, total acidity. Different letters in the same column indicate significant differences among genotypes at the 5% level, according to the LSD's multiple range test.

The alcohol content of wine is a consequence of the relative sugar content in grapes and varies depending on the variety of wine, as well as the winemaker [1,2]. As can be observed in Table 4, 'Calblanque' wine showed the lowest alcohol percentage as expected since it comes from a white variety whose wines usually have a lower alcohol content than those from red varieties. With respect to the rest of the wines, it can be observed how 'Calnegre' wine obtained the lowest alcohol percentage and 'Syrah' wine the highest. The rest of the wine varieties obtained intermediate values of alcohol content. It is remarkable that the wines of the new varieties obtained values of alcohol content lower than their parental wines. This could mean that our varieties could be an opportunity to obtain wines with a lower alcohol content in areas as warm as ours by allowing a coupling of phenolic and technological maturity and, at the same time, we could offer to the consumers, wines that are more adapted to their actual tastes.

All the wines studied showed values of total acidity ranging between 6 and 8 g/L of tartaric acid at the end of alcoholic fermentation (Table 4). The lowest value was found in 'Syrah' wines followed by 'Calblanque' wines and the highest value was found in 'Myrtia' wines, despite the fact that at the beginning of the winemaking process, all wines are adjusted to an acidity of 5.5 g/L with tartaric acid.

The pH of the wine is strictly connected with its microbiological and physicochemical stability [44] and it may contribute to the natural selection of microorganisms during winemaking [45,46]. Even the color of red wines may be strongly conditioned by the pH because this variable affects the equilibrium between the different forms of anthocyanins [47,48]. The pH level of a wine ranges from 3 to 4 [49]. The analyzed wines showed values ranging from 3.41 to 3.64, with 'Calblanque', 'Calnegre', 'Myrtia' and 'Monastrell' wines being those that reached the lowest values of pH; however, the highest value was found in 'Gebas' wine with a value of 3.64. In spite of the differences obtained in the different wines, values of pH around 3.6 are very adequate in warm areas such as ours.

Another parameter to take into account when we analyze the quality parameters of wines is relative density. The results showed how wines from parentals and the 'Calblanque' variety obtained the lowest relative density values; however, the rest of the wines from the new red varieties obtained the highest values, being statistically different with respect to the first.

Finally, the dry extract values correspond to all the non-volatile substances contained in it. The results in Table 4 showed data between 20.37 for 'Calblanque' wines and 32.61 for 'Calnegre' wines. As can be observed, the wines of the new red varieties obtained the highest values similar to relative density results.

3.4. Wine Spectrophotometric Characteristics

Spectrophotometric characteristics were evaluated in wines from the new varieties and their parentals at the end of alcoholic and malolactic fermentation. The results corresponding to color intensity, taint, anthocyanins, IPTs and different CIELab parameters are shown in Table 5.

Regarding color intensity (CI), differences among wines were very large. The CI values were higher at the end of alcoholic fermentation compared to those obtained at the end of malolactic fermentation. Due to the considerable chemical changes in the malolactic fermentation mainly driven by the increase in pH and the SO_2 addition, the color parameters were affected in the red wine. CI ranged between 60.28 ('Myrtia') and 14.68 ('Monastrell') at the end of alcoholic fermentation and between 40.02 ('Myrtia') and 11.69 ('Monastrell') at the end of malolactic fermentation. Wines from the parentals showed great differences with respect to the wines of the new varieties. With regards parental wines, 'Syrah' obtained the highest values followed by 'Cabernet Sauvignon' and 'Monastrell'. Regarding new varieties of wines, the wines with the highest CI were those from the 'Myrtia' variety, followed by 'Calnegre' and 'Gebas'. Different authors showed values of CI ranging between 3.65 and 25.7 at the end of malolactic fermentation in a study carried out in 'Monastrell' wines from different wineries from the same geographic area and, within each winery, from wines elaborated based on different market prices [50]. Values ranged from 13.1 to 21.3 in 'Monastrell' wines and 12.2–38.2 in 'Cabernet Sauvignon' wines at the end of alcoholic fermentation in a study carried out over two seasons [51]. As can be noticed with the results shown in Table 5, our new varieties are capable of producing wines with an extraordinarily high color despite being grown in areas with high temperatures and semi-arid conditions.

Table 5. Mean composition (2017–2021) of red wines at the end of alcoholic fermentation (AF) and malolactic fermentation (MF).

Parameters		Monastrell	Cabernet S.	Syrah	Calnegre	Gebas	Myrtia
Color intensity	AF	14.86 ± 0.52 a	19.17 ± 0.65 a	25.87 ± 2.56 b	46.66 ± 1.58 d	40.72 ± 2.31 c	60.28 ± 1.61 e
	MF	11.69 ± 1.70 a	16.00 ± 0.45 ab	18.66 ± 1.09 b	32.50 ± 2.46 d	25.99 ± 1.45 c	40.02 ± 1.97 e
Taint	AF	0.44 ± 0.02 a	0.44 ± 0.02 a	0.39 ± 0.01 a	0.38 ± 0.02 a	0.42 ± 0.03 a	0.39 ± 0.01 a
	MF	0.57 ± 0.03 b	0.56 ± 0.01 b	0.55 ± 0.02 b	0.48 ± 0.01 a	0.54 ± 0.01 b	0.48 ± 0.01 a
Anthocyanins	AF	571.00 ± 36.88 a	698.00 ± 52.84 a	1084.00 ± 58.57 b	1598.00 ± 54.40 c	1526.00 ± 90.92 c	1936.00 ± 252.68 d
	MF	330.47 ± 75.00 a	432.00 ± 54.00 a	692.00 ± 65.83 b	979.00 ± 110.38 c	972.00 ± 91.18 c	1262.00 ± 109.82 d
T.P.C.	AF	43.35 ± 1.86 a	45.10 ± 1.72 a	60.73 ± 3.60 b	94.41 ± 6.95 c	91.84 ± 7.99 c	100.78 ± 4.87 c
	MF	36.70 ± 3.99 a	41.84 ± 1.00 ab	53.83 ± 2.82 b	85.94 ± 8.07 c	82.73 ± 5.80 c	86.05 ± 3.70 c
L*	AF	13.77 ± 0.43 d	8.25 ± 0.81 c	3.91 ± 0.58 b	1.76 ± 0.16 a	1.35 ± 0.56 a	0.84 ± 0.20 a
	MF	14.18 ± 3.07 c	6.86 ± 0.67 b	5.03 ± 0.44 ab	2.57 ± 0.14 a	2.65 ± 0.34 a	1.46 ± 0.42 a
a*	AF	46.10 ± 0.51 e	38.43 ± 1.44 d	26.01 ± 2.55 c	12.74 ± 1.11 b	9.72 ± 4.00 ab	6.14 ± 1.47 a
	MF	44.89 ± 2.48 d	35.89 ± 1.49 c	31.30 ± 1.55 c	18.55 ± 1.03 b	18.91 ± 2.32 b	10.65 ± 3.08 a
b*	AF	23.69 ± 0.73 d	14.22 ± 1.40 c	6.74 ± 1.01 b	3.03 ± 0.27 a	2.33 ± 0.97 a	1.45 ± 0.35 a
	MF	19.15 ± 1.67 d	11.80 ± 1.14 c	8.66 ± 0.76 b	4.43 ± 0.25 a	4.58 ± 0.60 a	2.48 ± 0.69 a
C* (ab)	AF	51.84 ± 0.83 d	41.01 ± 1.82 c	26.88 ± 2.73 b	13.10 ± 1.14 a	10.00 ± 4.11 a	6.31 ± 1.51 a
	MF	48.87 ± 2.64 d	37.83 ± 1.77 c	32.48 ± 1.67 c	19.07 ± 1.08 b	19.46 ± 2.39 b	10.94 ± 3.16 a

Data expressed as mean value ± standard deviation. T.P.C., total phenolic content. Different letters in the same file indicate significant differences among genotypes at the 5% level, according to the LSD's multiple range test.

With respect to taint values at the end of alcoholic fermentation, they were similar for all wines, including those from parentals and new varieties, and indicated no oxidations in any of them. Nevertheless, at the end of malolactic fermentation, 'Calnegre' and 'Myrtia' showed the lowest values in comparison with the rest of wines, which showed upper values but were similar among them (Table 5).

Other phenolic parameters measured in wines were total anthocyanin and total phenolic compounds also measured previously in grapes at harvest. As can be observed in Table 5, the concentrations of anthocyanins shown in the wines of the new varieties were much higher than those shown by their parentals. Specifically, the 'Myrtia' wines, which were those with the highest anthocyanin content, doubled the content obtained by 'Syrah' wines and quadrupled that obtained by 'Monastrell' wines, both at the end of alcoholic and malolactic fermentation. The 'Calnegre' and 'Gebas' wines showed values of around 1500 mg/L of total anthocyanins, which were also higher than those obtained by the wines of their parentals ('Monastrell' and 'Cabernet Sauvignon'), although slightly less than in 'Myrtia' wines. It is known that the typical concentrations of free anthocyanins in full-bodied young red wines are around 500 mg/L, but can in some cases be higher than 2000 mg/L [52–54] as shown in the wines of the new varieties. Similar results were found in other works by different authors; Gil-Muñoz et al. (2018) [55], in a study carried out during two consecutive seasons, showed values of total anthocyanin in wines elaborated with varieties cross-bred from 'Monastrell' that ranged from 799.5 to 2206.4 mg/L during 2015 and from 1636.3 to 2210.2 mg/L in 2016; with the values reached being very different in function according to the analyzed season. In addition, Gil-Muñoz et al. (2021) [56], in an experiment carried out during three consecutive seasons in 'Monastrell' and 10 crossbreeds, showed how most of them had a higher anthocyanin concentration in wines with respect to 'Monastrell' wines, although differences were found between years.

Regarding IPTs, as was the case with anthocyanins, the final values were higher for the alcoholic fermentation than for malolactic fermentation, and they were also higher in wines from the new varieties compared to those from the parents. 'Syrah', 'Cabernet Sauvignon' and 'Monastrell' wines showed values of 60.73, 45.10 and 43.35 at the end of alcoholic fermentation, and 53.83, 41.84 and 36.70 at the end of malolactic fermentation, respectively. As can be observed, 'Monastrell' wines always obtained the lowest content of IPTs. With respect to the new varieties of wines, IPTs values ranged between 91.84 and 100.78 at the end of alcoholic fermentation and between 82.73 and 86.05 at the end of malolactic fermentation, with 'Myrtia' wines being those that obtained the highest values and 'Gebas' the lowest. 'Calnegre' showed intermediate values. Again, the results found in the wines of the new red varieties were quite a bit higher than those found in the wines of the parentals.

Finally, concerning the color measured at the end of alcoholic fermentation using the CIELab space (Table 5), the highest value of L* was reached by 'Monastrell' wines indicating they were the clearest wines, followed by 'Cabernet Sauvignon' wines and finally by 'Syrah' wines. New variety wines obtained lower L* values than parental wines, indicating that these wines were darker than parental wines; nevertheless, the lowest L* value was shown in 'Myrtia' wines, followed by 'Gebas' wines and finally by 'Calnegre' wines. With respect to final malolactic fermentation, the L* values increased slightly except in the case of 'Cabernet Sauvignon' wines, but the trend was similar to that found at the end of alcoholic fermentation. Regarding a* and b* parameters, which indicate red and yellow colors, respectively, different results were shown in the different wines analyzed. In general, values were lower at the end of alcoholic fermentation than malolactic fermentation, with the exception of 'Monastrell' and 'Cabernet Sauvignon' wines where the opposite happened. Paladines-Quezada et al. (2019) [51] showed L* values around 12 in 'Monastrell' wines and ranging between 4 and 11 in 'Cabernet Sauvignon' wines at the end of alcoholic fermentation. With respect to a*, the highest value was found in 'Monastrell' wines and the lowest in 'Myrtia' wines in the two moments analyzed at the end of the alcoholic and malolactic fermentation. With respect to b*, the highest value was found in

'Monastrell' wines, but the lowest results were found in the three new varieties of wines due to no statistical differences being found among them. The last parameter analyzed was C* (chroma), which is a parameter that indicates the contribution of a* (redness) and b* (yellowness), so values of C* close to or higher than 50 correspond to vivid colors. As expected, the results were similar to those obtained for parameters a* and b*, with higher values at the end of malolactic fermentation except for 'Monastrell' and 'Cabernet Sauvignon' wines. We were also able to observe the highest values in 'Monastrell' wines, and the lowest values for 'Myrtia' wines, although similar values were obtained at the end of alcoholic fermentation in the new varieties since no statistically significant differences were shown.

3.5. Wine Sensorial Analysis

The quality and phenolic characteristics of our wines from the new varieties were promising, but we wanted to check if they also had that quality sensorial characteristic, so we carried out a descriptive sensory analysis. The technique of descriptive analysis (DA) provides a quantitative analytical characterization of appearance, aroma, taste and mouthfeel as described in detail elsewhere [57,58].

As can be observed in Figure 2, 'Calblanque' wine was compared to 'Verdejo' wine due to both being white varieties, and in addition, 'Verdejo' is considered a high quality wine among white wines in Spain. Among the descriptive attributes taken into account, color, mouth and aroma characteristics were evaluated. Although the difference was small between both wines, the tasters were able to distinguish them sensorially, giving them different scores. With regard color, 'Calblanque' wines showed a higher value in comparison to 'Verdejo' wines. Regarding nose attributes, intensity was higher in 'Calblanque' wines than in 'Verdejo' wines; however, the quality was superior in the latter in comparison with 'Calblanque'. Intensity, quality and persistence in mouth were higher in 'Calblanque' than 'Verdejo' wines. Finally, harmony, a parameter that alludes to the global perception of the wine, also scored higher in 'Calblanque' wines. Moreno-Olivares et al. (2020) [59], in a study carried out with different white crossbreeds from 'Monastrell', showed results that highlighted how the crosses MT103, MC69 and MC180 ('Calblanque') showed significant differences from and better quality than the 'Verdejo' wine.

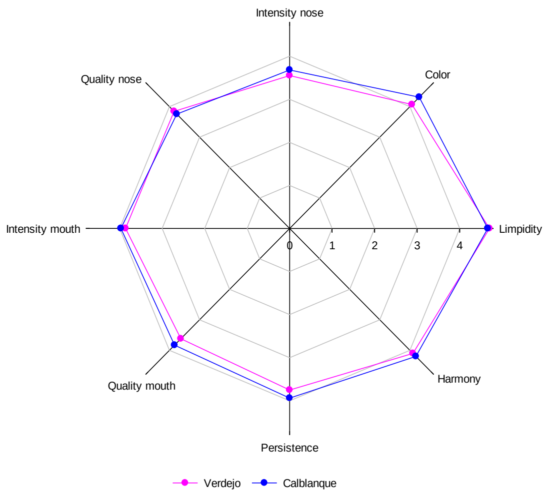

Figure 2. Sensorial analysis of 'Verdejo' and 'Calblanque' wines at the end of malolactic fermentation.

Figure 3 shows the sensorial analysis of the parentals and the new red varieties wines. As can be observed, more differences were found between the new varieties and parental wines. Regarding color, the highest scores were shown in 'Myrtia', 'Calnegre' and 'Gebas' wines. Limpidity was also highest in 'Myrtia' wines, although similar scores were shown in 'Monastrell', 'Syrah', 'Calnegre' and 'Gebas' wines, with 'Cabernet Sauvignon' being the variety with the lower score. With respect to the nose attributes, similar and the highest intensities were found in 'Syrah', 'Myrtia' and 'Calnegre' wines, intermediate values were shown in the 'Monastrell' and 'Gebas' wines, and finally, again, 'Cabernet Sauvignon' wines showed the lowest score. The highest quality nose was found in 'Syrah' and 'Calnegre' wines followed by 'Myrtia' and 'Gebas' and finally by 'Monastrell' and 'Cabernet Sauvignon' wines. It is known that the aromatic profile of many wines depends on the varietal compounds of the grapes that have been employed in their production. As well as lactic acid, the main substrate of malolactic fermentation, during this fermentation, there are a large number of metabolic end products, produced by specific bacterial species/strains that are responsible for modifying the aroma and flavor perception of wine [60]. With respect to mouth characteristics, the highest intensity, quality and persistence was shown by the new varieties and 'Syrah' wines, with the lowest score shown by 'Monastrell' and 'Cabernet Sauvignon' wines. Finally, harmony was superior in 'Syrah' wines, followed by the rest of the new varieties, and then by 'Monastrell' and 'Cabernet Sauvignon' wines.

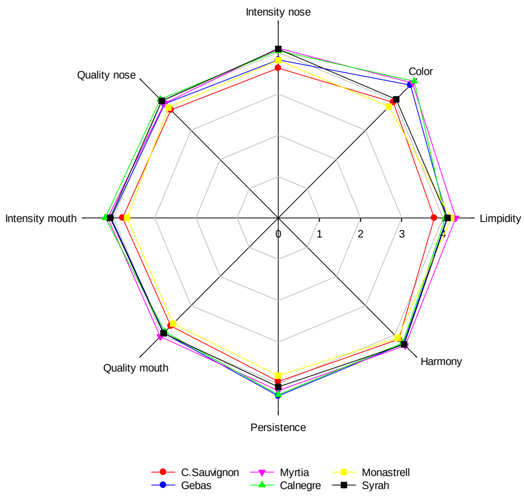

Figure 3. Sensorial analysis of 'Myrtia', 'Gebas', 'Calnegre', 'Syrah', 'Cabernet Sauvignon' and 'Monastrell' wines at the end of malolactic fermentation.

4. Conclusions

Crossbreeding programs generate great genetic variation and allow the selection of new genotypes, as described in this work, that are better adapted to the specific conditions of the viticulture zone. The attributes of the white variety 'Calblanque', and the red varieties 'Calnegre', 'Gebas' and 'Myrtia', registered by the IMIDA as commercial varieties and authorized for winemaking in the Region of Murcia, could allow their better adaptation to the effects of high temperatures on grape and wine quality in semi-arid areas. The red genotypes were selected for their phenolic quality—which was very superior to that of the parentals—and the white variety 'Calblanque' was selected for its good balance of acidity and aromatic profile. From a sensorial point of view, the new varieties of wines also showed high scores in comparison with their parentals.

Therefore, the new varieties described in this work represent a support to the wine sector of the area, which will have an innovative and competitive material of high quality, while maintaining the Mediterranean profile of the wines made with these varieties.

5. Patents

The new varieties are protected at the European level. The ownership and all rights over them belong to IMIDA. The breeders are: Adrián Martínez-Cutillas, José Ignacio Fernández-Fernández, Leonor Ruiz-García, Celia Martínez-Mora, Juan Antonio Bleda-Sánchez and Rocío Gil-Muñoz.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070760/s1, Table S1: Cumulative and average values of meteorological parameters recorded in the experimental farm from the starting to the end of the breeding program (1997-2021); Table S2: Cumulative and average values of meteorological parameters recorded in the experimental farm during the evaluation period (2017-2021); Table S3: Ampelographic characteristics of the parental and new varieties indicated with the UPOV descriptors (2008) and the CPVO technical protocol (CPVO-TP/050/2) for distinctness, uniformity and stability tests.

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