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# Characterization of Local Mediterranean Grapevine Varieties for Their Resilience to Semi-Arid Conditions under a Rain-Fed Regime

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**Abstract:** Viticultural adaptations to climate change are needed, and the utilization of grapevine varieties that are better-adapted to water scarcity could contribute to finding grape varieties that are adapted to climate change. The present research was carried out to expand the limited knowledge on the minor varieties Arcos and Forcallat in comparison with three other more widespread traditional Mediterranean cultivars (Bobal, Garnacha, and Monastrell). An ampelographic characterization was carried out and provided with the characteristics for the cv. Arcos, which have not been previously described, as well as traits that are useful for differentiating it from the cv. Forcallat. Both varieties maintained low stomatal conductance, having the highest number of small stomata in comparison to the rest of varieties. Arcos and Forcallat also showed the highest intrinsic water use efficiency in addition to being late ripening, a characteristic that could be of interest in the context of water scarcity and warm climates for better coupling of technological and phenolic maturity. In parallel, we analyzed Veremeta plants considered a synonym of Monastrell, which were growing in the same field. The synonymy was confirmed by SSR markers, but phenotypic differences between plant materials were determined in relation to their ampelographic, agronomical, and physiological traits. Indeed, both accessions are very interesting as materials to be studied in agronomic trials under different watering regimes in order to deepen our understanding of the mechanisms underlying the drought tolerance of the evaluated Mediterranean varieties.

**Keywords:** water status; ripening; drought; stomata; intrinsic water use efficiency



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## 1. Introduction

There is great concern about the consequences of climate change and anthropogenic global warming on grapevine cultivation, particularly in the Mediterranean area [1]. The expected erratic precipitation patterns and increased temperatures will make drought events more frequent. During droughts, photosynthesis can be limited by stomatal closure and the impairment of photosynthetic machinery. Non-stomatal limitations have been shown to occur below 50 mmol $m^{-2}s^{-1}$ on grapevines [2]. Under conditions of severe water deficits ($\Psi$stem < −1.6 MPa), turgor loss and xylem cavitation can lead to leaf shedding and even grapevine mortality [3]. These changes do not affect all cultivation areas the same, but they may lead to the establishment of vine cultivation in novel areas in northern latitudes in Europe, the replacement with other crops in other localizations, and varietal and cultivar substitution by cultivars adapted to the novel conditions imposed by climate change. In this scenario, the Mediterranean has been classified as one of the most responsive regions [4]. In addition, in the area where grapevines have been traditionally cultivated under rain-fed conditions, a steady increase in vineyard irrigation as a way to overcome severe drought stress and ensure more regular and predictable yields has been established [5]. However, water is a scarce resource, and the optimization of water use is a necessity. As of today, improving WUE (Water Use Efficiency) is a key topic for viticulture sustainability [6], which can be achieved considering the following: (i) the agronomic techniques related to vineyard management to obtain water savings with deficit irrigation and improved soil management, and (ii) the genetic resources focused on the selection of cultivars, clones, or rootstock–scion combinations with a higher WUE. For the latter, there is a need to identify cultivars and/or select drought-tolerant crop varieties and/or clones, as well as to know the differences in drought tolerance among existing rootstocks and grapevine varieties [3,7–9].

To characterize the behavioral differences under drought stress among grapevine genotypes, many studies have focused on agronomic indicators such as yield and grape quality, while other studies have focused on fine-tuning physiology such as stomatal regulation and carbon assimilation [3]. Stomata are key players in a plant's response to drought, given their tendency to close under drought conditions to reduce transpiration to therefore avoid critical stem water potential and conserve water. Stomatal conductance (gs) can be considered as an integrative parameter that reflects the water stress experienced by plants [10]. It has been reported that stomatal density is both varied and characteristic of different grapevine cultivars grown under the same conditions, and it is considered an evolutionary adaptation rather than a short-term avoidance mechanism [11]. High stomatal density coupled with lower stomatal dimensions are features that minimize transpiration and can also be considered adaptations of the cultivars to water stress [12,13].

In the present study, we propose to enrich the knowledge of Mediterranean grapevine varieties growing in semi-arid conditions under a rain-fed regime in an old vineyard of eastern Spain by analyzing their agronomic, physiological, and anatomical traits related to their resilience to semi-arid conditions. We ampelographically described, for the first time, the minor variety Arcos and compared it with Forcallat, another late-ripening minor variety with which it is sometimes confused. Ampelographic analysis was also performed in Veremeta Clara, a variant of the autochthonous variety Monastrell, in order to determine the differences between both of them, as well as among plants of the varieties Bobal and Garnacha growing in a mature commercial vineyard.

## 2. Materials and Methods

### *2.1. Plant Material, Varietal Identification, and Growing Conditions*

The experimental field was a ~50 years-old vineyard located in Biar (Plot 12–248; 38°39′05.3″ N 0°48′05.9″ W), Alicante, Spain, planted in 1973. Plants of the cv. Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta were randomly grown in the same plot/vineyard, as done previously in old vineyards with a 2 m × 2 m planting frame, which is widely used in old dryland fields on the Iberian Peninsula. The field was managed under organic farming and rain-fed conditions. Plants were grafted onto a 41B rootstock. The vineyard training system was an open-vase system without a supporting structure, traditionally used in dry Mediterranean climate conditions. This conduction system is known as 'Gobelet' in France and 'Alberello' in Italy. The type of pruning in the studied field was 'short' (with 2–3 buds per spur), and the total load of buds did not exceed 16–20, depending on the development (number of branches) of the plant.

Varietal confirmation was performed in all the evaluated plants using a multiplex PCR with SSR markers (VVS2, VVMD5, VVMD6, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79, VrZAG64, VrZAG83, and VMC11b11) as described by [14].

*2.2. Climatic Conditions*

The climatic conditions in the experimental area were those typical of the inland Mediterranean Sea basin with hot and dry summers. The average values from the last 10 years for annual mean temperatures, relative humidity, atmospheric pressure, and wind speed in the experimental area were 15.3 °C; 66%, 1017.5 hPa, and 5.1 km/h, respectively. The average maximum temperature was 40.2 °C and the minimum −5.4 °C. For the experimental seasons, the average of the maximum and minimum temperature as well as precipitation are reported for each month and year in Figure S1. The range of the accumulated annual precipitation for this period varied from 305 to 506 mm (Table S1). The average for minimum and maximum humidity was 36.4% and 96.3%, with it being lower in summer. Although in general the wind speed was low and the precipitation scarce, on some days gusts of wind reached 53–87 kmh$^{-1}$ and a maximum daily precipitation of around 50 mm was registered.

*2.3. Ampelographic Characterization of Varieties*

The ampelographic characterization of the cv. Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta was performed following the Organisation Internationale de la Vigne et du Vin descriptors [15]. The descriptors are as follows. For young shoots: intensity of anthocyanin coloration on prostrate hairs of tip (003) and density of prostrate hairs on tip (004). For young leaves: color of the upper side of blade (051) and density of prostrate hairs between main veins on lower side of blade both in the 4th leaf (053). For mature leaves: shape of blade (067), number of lobes (068), shape of teeth (076), degree of opening/overlapping of petiole sinus (079), shape of base of petiole sinus (080), density of prostrate hairs between the main veins on lower side of blade (084), and depth of upper lateral sinuses (094). For bunches: length (peduncle excluded) (202), width (203), density (204), length of peduncle of primary bunch (206), shape (208), number of wings of the primary bunch (209), and weight of a single bunch (502). For berries: length (220), width (221), shape (223), color of skin (225), particularity of flavor (236), length of pedicel (238), length of seeds (242), weight of seeds (243), and single berry weight (503). This ampelographic descriptors were determined for the six varieties under study during 2020 and 2021. In addition, a colorimeter (Konika Minolta) was used to measure leaf color on the upper and lower sides of leaves using CIELAB color space. The results were recorded using the color parameters L (the lightness of the color (0 = black, 100 = white)), a (the red (positive)/green (negative) coordinate), and b (the yellow (positive)/blue (negative) coordinate). The total color difference (AE) between Monastrell and Veremeta was obtained using the formula:

$$\sqrt{(\Delta L2 + \ \Delta a2 + \ \Delta b2}$$

where ΔL, Δa, and Δb are differences from L, a, and b between the two varieties (Monastrell and Veremeta). Two measurements per leaf in ten plants per variety were made.

*2.4. Vigour and Other Related Agronomical and Quality Traits*

Growth is measured as vigor, estimated with a visual index from 1 to 4 (from lowest to highest leafiness), vine height, and width, which were measured in ten plants from each of the six studied varieties. Budburst, veraison, and harvest dates were noted.

Total soluble solids (TSS) were measured in five representative bunches each from five plants by mixing berries from different parts of the bunch on 14 September 2021 using a refractometer (PR-101 Series Palette, Atago Co. LTD, Tokyo, Japan). A sample of 100 berries from each bunch was used for determining titratable acidity (TA) and pH with an automatic titrator (Metrohm, Herisau, Switzerland). A sample from 50 berries was homogenized with a blender (Ultraturrax T25, IKA-Werke GmbH &Co. KG, Freiburg, Staufen, Germany) to assess the phenolic and anthocyanin content via UV/VIS spectrophotometry (Lambda 35, Perkin Elmer Inc., Waltham, MA, USA) following the standard methods as reported by [16] from the Australian Wine Research Institute. In addition, the concentration of grape anthocyanins in relation to the TSS levels was calculated.

*2.5. Stomatal Conductance and Other Gas Exchange Measurements*

In 2020 and 2021, stomatal conductance (gs) was recorded throughout the growing season in fully sunlight-expanded leaves in five plants per variety from each of the six studied varieties using a leaf porometer (SC-1, Meter, Munich, Germany), determining gs in the third or fourth leaf (with similar development) from the end of the vine tips. One measurement per plant in five plants per variety was carried out on 16 and 27 July 2020 (a.m.) and on 10 August 2020 (at a.m. and p.m.). Three measurements per plant were taken on 6 July 2021 and 28 July 2021 (at am and pm). In addition, gs, leaf net photosynthesis (An), transpiration rate (E), and leaf internal $CO_2$ concentration (Ci) were determined in two leaves per plant in five plants per variety on 20 August 2021 and 10 September 2021 using an infrared open gas exchange system (Li96400 Licor Inc. Lincoln, NE, USA) equipped with a 2 $cm^2$ chamber at saturated light (1500 µmol $m^{-2}$ $s^{-1}$) and at $CO_2$ concentrations of 400 $\mu mol^{-1}$ air. The ratio An/gs, referred to as WUEi (Intrinsic Water Use Efficiency) [17], was also calculated. In addition, the plant water status was estimated by measuring stem water potential (Ψstem) at midday using a Scholander pressure chamber (PMS 600, PMS Instrument Company, Albany, OR, USA). Prior to measurement, the leaves were bagged with plastic film and aluminum foil for at least 1 h.

*2.6. Stomatal Characterization*

For the study of anatomical traits, fresh adult leaves of similar size, age, and exposure, harvested on 28 July 2021, were used. Three leaves per plant and five plants per variety were taken from each of the six varieties studied. Abaxial epidermal replicas were prepared with the help of a transparent nail polish, collected from the lower side of all leaf samples. After 10 min of drying, sticky tape was used to peel off each polished area, after which it was pressed onto a microscopic slide as described by [18]. Two photomicrographs were taken at 400× magnification of each microscopic slide. For this purpose, a Leica DM750 microscope with ICC50 W camera module (Leica, Wetzlar, Germany) was employed. The area of the photomicrographs was 0.06954 $mm^2$ (0.305 mm × 0.228 mm). The following quantitative variables were directly measured in thirty 400× photomicrographs per variety: total number of stomata, length of at least 10 stomata, and total number of epidermal cells. Stomatal length was recorded with the Image J program [19]. The number of stomata and total number of epidermal cells were counted independently by three different operators and subsequently agreed upon. The individual value of each measurement was transformed to an area unit of 1 $mm^2$. The following parameters were also determined: epidermal cell area ($\mu m^2$) and stomatal index, calculated as the amount of stomata/number of cells in the same area ×100 according to [20].

*2.7. Statistical Analysis*

A simple ANOVA (analysis of variance), including variety as the factor, was carried out for vigor and other related agronomical and quality traits, as well as for stomatal characterization. The gs and other gas exchange measurements were analyzed as the factorial ANOVA, including the variety and the day or time of measurement and their interaction as effects. The least square differences (LSD) multiple range test was used to determine which means were significantly different ($p < 0.05$). Correlations between traits were also calculated. All analyses were carried out using the Statgraphics plus software (5.1 for Windows, 1994, Statistical, Corporation, Warrenton, VA, USA).

## 3. Results

The evaluations were carried out throughout 2020 and 2021 to obtain new insights on the varieties Arcos, Bobal, Garnacha, Forcallat, Monastrell, and Veremeta growing under rain-fed conditions and organic farming practices in an old vineyard in Alicante. This evaluation included the following: (i) an ampelographic characterization (including the variety Arcos for the first time), (ii) the characterization of the specific accessions of the rest of the varieties under study, all of which were red wine making varieties, and (iii) a comparison of Monastrell and Veremeta, as they are synonymies, as indicated by their SSR profiles (Table S2). Measurements of gs and other related parameters, as well as a stomata characterization, were also carried out.

### *3.1. Ampelographic and Agronomical Related Traits*

The ampelographic traits for Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta varieties are showed in Table 1. A photographic description for the cv. Arcos is also shown in Figure 1, as it was the first time this cultivar has been described. Arcos had a high density of prostrate hairs with a low intensity in anthocyanin coloration at the tip, and young bronzed leaves with cottony undersides. Regarding the mature leaves, they were penta-lobulated, pentagonal in shape, their upper surface was dark, and they had a cottony underside with the presence of prostrate hairs between the main veins, also showing straight and convex teeth and shallow closed overlapping brace-shaped lateral sinuses. The bunches were conical and had long peduncles with a medium density and weight (390 g). Regarding the berries, they had a blue-black color with a globose shape, herbaceous flavor, and single averaged fresh weight of 1.89 g (Table 1, Figure 1).


**Figure 1.** Images from Arcos grapevine variety: conical bunches (**A**,**E**); tip and young leaves (**B**); vine growth (**C**,**D**); mature leaves (**F**,**G**); blue-black globose berries (**H**); seeds (**G**).

**Table 1.** Ampelographic traits of the varieties Arcos, Bobal, Forcallat, Garnacha, and Monastrell and its variant Veremeta growing in an old vineyard of Biar.

| | **Arcos** | **Bobal** | **Forcallat** | **Garnacha** | **Monastrell** | **Veremeta Clara** |
|---|---|---|---|---|---|---|
| Young shoot | | | | | | |
| OIV [1] 003 | 3 | 1 | 1 | 5 | 3 | 3 |
| OIV 004 | 7 | 7 | 7 | 1 | 9 | 9 |
| Young leaf | | | | | | |
| OIV 051 | 3 | 2 | 2 | 3 | 3 | 3 |
| OIV 053 | 5 | 7 | 7 | 1 | 7 | 7 |
| Mature leaf | | | | | | |
| OIV 067 | 3 | 4 | 4 | 3 | 3 | 3 |
| OIV 068 | 3 | 3 | 4 | 3 | 3 | 3 |
| OIV 076 | 5 | 3 | 2 | 2 | 2 | 2 |
| OIV 079 | 5 | 5 | 3 | 3 | 5 | 3 |
| OIV 080 | 3 | 3 | 1 | 1 | 5 | 1 |
| OIV 081-2 | 1 | 1 | 1 | 1 | 1 | 1 |
| OIV 084 | 5 | 7 | 7 | 1 | 7 | 7 |
| OIV 087 | 1 | 3 | 1 | 1 | 1 | 1 |
| OIV 094 | 5 | 5 | 7 | 3 | 1 | 3 |
| Bunch | | | | | | |
| OIV 202 | 7 [234] (210–260) | 5 [182] (170–190) | 7 [226] (220–230) | 3 [130] (120–150) | 5 [166] (150–180) | 7 [196] (180–230) |
| OIV 203 | 5 [128] (110–140) | 5 [134] (130–140) | 5 [144] (130–160) | 3 [96] (80–110) | 5 [128] (120–140) | 5 [120] (110–140) |
| OIV 204 | 5 | 7 | 5 | 7 | 7 | 5 |
| OIV 206 | 3 [40] (40–10) | 1 [16] (10–20) | 3 [36] (30–40) | 1 [18] (10–20) | 1 [18] (10–30) | 1 [24] (20–30) |
| OIV 208 | 2 | 2 | 2 | 3 | 2 | 2 |
| OIV 502 | 5 [390] (300–450) | 5 [417] (355–483) | 5 [394] (320–450) | 3 [177] (133–266) | 3 [258] (230–287) | 3 [218] (180–288) |
| Berry | | | | | | |
| OIV 220 | 3 [13.4] (13.0–14.0) | 3 [16.6] (16.0–17.0) | 3 [16.3] (16.0–17.0) | 3 [14.7] (14.0–15.0) | 3 [14.4] (13.0–15.0) | 3 [11.8] (11.0–13.0) |
| OIV 221 | 3 [13.2] (13.0–14.0) | 3 [15.8] (15.0–16.0) | 3 [13.2] (13.0–14.0) | 3 [13.3] (13.0–14.0) | 3 [12.6] (12.0–13.0) | 3 [12.6] (12.0–13.0) |
| OIV 223 | 2 | 2 | 3 | 2 | 2 | 2 |
| OIV 225 | 6 | 6 | 6 | 5 | 6 | 6 |
| OIV 236 | 4 | 4 | 4 | 1 | 1 | 4 |
| OIV 242 | 5 [5.3] (5.0–5.5) | 7 [7.1] (7.0–7.5) | 5 [5.3] (5.0–5.5) | 5 [5.1] (5.0–5.5) | 5 [5.2] (5.0–5.5) | 5 [5.8] (5.5–6.0) |
| OIV 503 | 1 [1.89] (1.80–1.90) | 3 [3.07] (3.00–3.20) | 1 [1.89] (1.80–1.90) | 1 [1.73] (1.70–1.80) | 1 [1.97] (1.90–2.00) | 1 [1.65] (1.60–1.70) |

[1] OIV value [mean] (minimum–maximum).

Ampelographic differences were found between Monastrell and Veremeta, despite them showing the same SSR profile (Tables 1 and S2). Specifically, they differed in the ampelographic leaf descriptors 079 (degree of opening overlapping petiole sinus), 080 (shape of base of petiole sinus), and 094 (depth of upper lateral sinuses), as well as 202 (bunch length) 204 (density) and 236 (berry flavor). Monastrell had a higher number of leaves with overlapped sinuses and with brace-shaped petiole sinuses, whereas a higher amount of non-overlapped leaves with wider sinuses were found in Veremeta (Figure 2A,B). Monastrell also had the shortest and most compact bunches with grapes with a non-particular flavor that were more herbaceous in Veremeta. Leaf color also differed between Veremeta and Monastrell, mainly on the upper side of the leaf, with a difference of

2.96 for AE, which indicates a slightly lighter leaf in Veremeta. Both varieties had an upright growth, with Veremeta being more vigorous than Monastrell (Figure 2C,D).

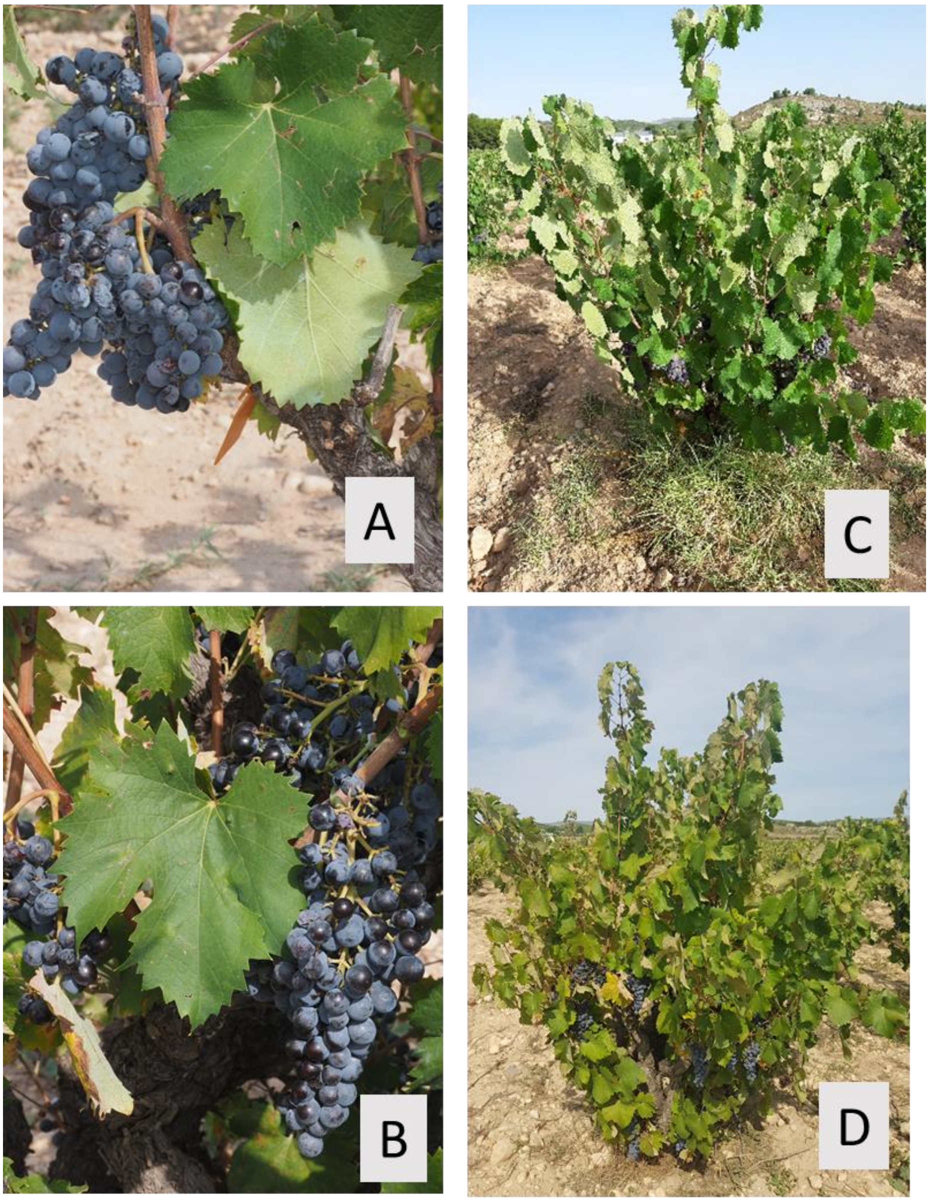


**Figure 2.** Bunches, leaves, and vines of cv. Monastrell (**A**,**C**) and Veremeta (**B**,**D**).

Forcallat had circular hepta-lobulated leaves, with straight teeth and a petiole with a U-shaped sinus and lateral sinuses that were very marked and deep. Its bunches were conical and medium in size with broad ellipsoid berries of an herbaceous flavor (Table 1). It showed an upright growth (Figure S2B). Forcallat leaves had the highest L (luminosity) and b (blue–yellow opponents) on both sides of the leaf with respect to all the other varieties (Table S3). Garnacha had the highest intensity coloration on the prostrate hairs of the tip and smaller bunches in comparison to the other varieties. Its bunches were funnel-shaped and had berries with a non-particular flavor (Table 1). Garnacha had a high vigorous growth (Figure S2C) and high fertility (data not shown). All the assayed varieties, with the exception of Garnacha, had leaves with a cottony underside with prostrate hairs between the veins of the leaves. Bobal stood out by the erect hairs on the veins on the undersides of its leaves and the weight of its clusters, which had the biggest grapes. Its bunches were compact, similar to those of Garnacha and Monastrell (Table 1, Figure S4).

Regarding phenology, budburst for all the evaluated varieties began on 10–20 April, with veraison starting at the end of July–early August, with a little delay in Forcallat and mainly in Arcos (Figure S4). Both of these varieties were late ripening. On 24 September 2021, the grapes from Arcos and Forcallat had an average of 15–16° Brix, whereas 21–23° Brix was measured in Garnacha and Veremeta, the two earlier varieties. Intermediate ripening was observed in Bobal and Monastrell, with averaged values of 18.5 and 19.2° Brix, respectively. Therefore, Veremeta and Monastrell also differed in ripening. Similar total acid concentrations were found in Bobal, Forcallat, Garnacha, and Monastrell, which showed lower values than Arcos and Veremeta. The highest anthocyanin content was found in Veremeta (1.59) and the lowest in Forcallat and Garnacha (close to 0.70). Intermediate values (1.1–1.2) were noted in Arcos, Bobal, and Monastrell. Varieties with a low total phenolic index (Forcallat and Garnacha) also had lower concentrations of total berry anthocyanins and a lower anthocyanin/Brix ratio (Table 2). The accumulation of total phenolic index and anthocyanins was expected to increase in Arcos and Forcallat, as these are late-ripening varieties.

**Table 2.** Agronomic- and quality-related traits for Arcos, Bobal, Garnacha, Forcallat, Monastrell, and Veremeta varieties.

| Variety | VI | VH | VW | WB | TSS | pH | TA | TPI | A | A/TSS |
|---|---|---|---|---|---|---|---|---|---|---|
| Arcos | 2.4 bc | 71 a | 110 bc | 144 a | 16.4 a | 3.5 | 4.74 b | 3.3 b | 1.09 b | 0.066 |
| Bobal | 3.6 de | 85 b | 199 d | 264 b | 18.5 b | 4.2 d | 3.14 a | 3.0 b | 1.16 c | 0.063 |
| Forcallat | 2.0 b | 68 a | 76 a | 142 a | 15.4 a | 3.9 bc | 3.48 a | 2.5 a | 0.70 a | 0.046 |
| Garnacha | 4.0 e | 153 d | 207 d | 153 a | 21.3 c | 4.2 d | 3.56 a | 2.2 a | 0.69 a | 0.032 |
| Monastrell | 1.2 a | 63 a | 124 c | 147 a | 19.2 b | 3.8 b | 3.23 a | 3.1 b | 1.20 d | 0.063 |
| Veremeta | 3 cd | 133 c | 98 b | 158 a | 23.1 c | 3.9 c | 4.88 b | 3.2 b | 1.59 e | 0.069 |

VI: vigor index; VH: vine height (cm); VW: vine with (cm); WB; weight of 100 berries (g); TSS: total soluble solids (° Brix); TA: total acids (g/L); TPI: total phenolic index (mg/g); A: anthocyanins (mg/g). Mean values within a column separated by different letters are different ($p < 0.05$).

### *3.2. Evaluation of Stomatal Conductance (GS) and Related Parameters*

Stomatal conductance was recorded in both seasons in the leaves of the varieties Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta with a porometer. In general, higher gs values were found in 2020 than in 2021 (Table 3) probably because in 2020, rainfall was higher than in the following season (Table S1.) Data from 2020 indicated that in the period analyzed (from 16 July 2020 to 10 August 2020), Arcos and Forcallat showed the lowest gs, whereas Veremeta had the highest, differing from Monastrell (Table 3). On average, Veremeta also had a higher leaf conductance than Garnacha and Bobal, both with higher gs values with respect to Arcos and Forcallat. Similar gs values were observed among the measurement dates in each variety, and only a reduction in gs was observed in Forcallat on 10 August 2020. On this date, gs was also noted in the afternoon (p.m.), with a greater reduction in gs in Arcos and Forcallat with respect to the other varieties. Stomatal conductance throughout the season showed a similar behavior in both years: Arcos and Forcallat showed the lowest gs, and Garnacha and Veremeta the highest. The am and pm measurements on 6 and 28 July 2021 indicated that, in general, varieties closed their stomata in the pm, but at different degrees depending on the variety and gs values recorded in the am. A high reduction was noted in Forcallat and Arcos on different dates, whereas lower reductions were found in Garnacha.

**Table 3.** Leaf stomatal conductance (μmol$H_2O$m$^{-2}$s$^{-1}$) in plants of Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta during the summers 2020 and 2021.

| | | | 2020 | | |
|---|---|---|---|---|---|
| **Variety** | **16 July (a.m.)** | **27 July (a.m.)** | **10 August (a.m.)** | **10 August (p.m.)** | **% R** |
| Arcos | 277 a 1 | 279 a 1 | 282 ab 1 A | 172 a A | 38.5 |
| Bobal | 377 ab 1 | 430 bc 1 | 438 bc 1 A | 424 b A | 0.0 |
| Forcallat | 369 ab 2 | 366 ab 2 | 247 a 1 A | 146 a A | 55.5 |
| Garnacha | 405 ab 1 | 413 bc 1 | 388 abc 1 A | 380 ab A | 5.5 |
| Monastrell | 408 b 1 | 391 b 1 | 348 abc 1 A | 282 ab A | 26.4 |
| Veremeta | 587 c 1 | 504 c 1 | 530 c 1 B | 411 b A | 23.9 |
| Mean | 404 | 397 | 372 A | 302 A | 28.8 |

| | | | | 2021 | | | | |
|---|---|---|---|---|---|---|---|---|
| **Variety** | **6 July** | **11.00 a.m.** | **15.00 p.m.** | **% R** | **28 July** | **11.00 a.m.** | **15.00 p.m.** | **% R** |
| Arcos | 150 a | 152 a A | 149 a A | 2.5 | 168 b | 188 b B | 148 ab A | 21.3 |
| Bobal | 255 c | 272 c A | 238 c A | 12.8 | 206 d | 222 c B | 190 c A | 14.2 |
| Forcallat | 158 a | 176 a B | 139 a A | 21.3 | 141 a | 150 a A | 132 a A | 12.3 |
| Garnacha | 225 b | 237 bc A | 212 bc A | 10.9 | 207 d | 204 bc A | 211 c A | 0.0 |
| Monastrell | 222 b | 242 bc B | 202 b A | 16.7 | 184 c | 205 bc B | 164 b A | 20.4 |
| Veremeta | 212 b | 223 b A | 200 b A | 10.3 | 229 e | 249 d B | 209 c A | 16.3 |
| Mean | 204 | 217 B | 190 A | 12.7 | 189 | 203 B | 176 A | 14.1 |

% R: % Reduction (p.m. vs. a.m.). Mean values within a column separated by different lowercase letters are different ($p < 0.05$). Mean values between hours separated by different uppercase letters are different ($p < 0.05$). Mean values between files separated by different numbers are different ($p < 0.05$). Data obtained with a porometer (SCI-meter).

On 20 August and 10 September, plant water status (Ψstem) measurement indicated that Ψstem differed among the varieties and plants. Values ranged from −1.13 (in Veremeta) to −1.61 (in Forcallat) on 20 August, and from −0.98 (in Veremeta) to −1.49 (in Forcallat) on 10 September (Table 4). On these dates, gs and other gas exchange parameters (An, Gs, Ci, and E) were also recorded using an infrared open gas exchange system. In August, the lowest values for An, gs, Ci, and E were recorded in Arcos and Forcallat, which showed the highest WUEi. Contrary to this, Garnacha and Veremeta had the highest An, gs, and E values, and the lowest WUEi. On 10 September, Bobal and Monastrell did not differ from Arcos and Forcallat in either An, gs, Ci, E, or WUEi.

In the experimental conditions, a decrease in gs, E, and Ci was observed for all the varieties on 10 September with respect to 20 August. In contrast, An was maintained. In spite of this, a high correlation was obtained for An and gs (0.93 on 20 August; 0.91 on 10 September), as well as for An and E (0.94 on 20 August; 0.81 on 10 September). Regarding WUEi, it was negatively correlated with An (−0.79 and −0.66, respectively), gs (−0.87 on both dates), and Ci (−0.99 on both dates). Figure S5 shows gs vs. Ψstem values on 20 August. On this date, all the Arcos and Forcallat plants had very low Ψstem, whereas the range for Ψstem in the rest of varieties ranged from 1.1 to 1.5 in Bobal and Monastrell and −0.9 to −1.4 in Garnacha and Veremeta. Garnacha and Bobal showed the highest slopes.

**Table 4.** Determination of midday stem water potential (Ψstem) and leaf gas exchange related parameters in 2021.

| Varieties | $\Psi_{Stem}$ | An | GS | Ci | E | WUEi |
|---|---|---|---|---|---|---|
| | | | **20 August** | | | |
| Arcos | −1.53 a | 6.67 a | 70.3 a | 209.67 a | 1.97 a | 102.27 d |
| Bobal | −1.35 b | 10.09 b | 153.4 b | 256.10 b | 3.56 b | 70.37 bc |
| Forcallat | −1.61 a | 6.98 a | 75.2 a | 217.33 a | 2.02 a | 97.37 d |
| Garnacha | −1.14 c | 13.73 c | 269.7 c | 276.20 c | 5.55 c | 53.53 a |
| Monastrell | −1.40 b | 9.94 b | 126.3 ab | 239.78 b | 2.93 b | 80.84 c |
| Veremeta | −1.13 c | 15.59 c | 251.8 c | 257.00 b | 5.40 c | 64.04 ab |
| Mean | −1.36 | 10.5 | 157.78 | 242.68 | 3.58 | 78.07 |
| | | | **10 September** | | | |
| Arcos | −1.48 a | 6.09 a | 47.0 a | 165.00 a | 1.10 a | 133.65 c |
| Bobal | −1.12 bc | 10.42 ab | 100.7 ab | 189.67 ab | 1.85 a | 114.80 bc |
| Forcallat | −1.49 a | 8.68 a | 68.4 a | 163.50 a | 1.62 a | 131.33 c |
| Garnacha | −0.99 cd | 14.58 c | 167.1 c | 213.40 bc | 3.01 b | 94.77 ab |
| Monastrell | −1.18 b | 10.47 ab | 104.7 ab | 193.14 ab | 1.80 a | 112.72 bc |
| Veremeta | −0.98 d | 13.32 bc | 161.1 bc | 229.00 c | 2.28 ab | 88.00 a |
| Mean | −1.21 | 10.6 | 108.17 | 192.29 | 1.94 | 112.55 |

An: photosynthesis; gs: stomatal conductance (μmol$H_2O$m$^{-2}$s$^{-1}$); Ci: intercellular $CO_2$ concentration (μmol mol$^{-1}$); E: transpiration rate (mmol$H_2O$m$^{-2}$s$^{-1}$); WUEi: intrinsic water use efficiency (μmol$CO_2$mol$H_2O$m$^{-2}$s$^{-1}$). Mean values within a column separated by different lowercase letters are different ($p < 0.05$). Leaf gas exchange data were obtained with an infrared gas analyzer (Li 96400 Licor).

### 3.3. Stomata Densities and Size

The leaf stomata densities varied among the varieties, ranging from 89 to 460 stomata mm$^{-2}$, with an average of 242.6. On average, Forcallat had the highest density with 280 stomata mm$^{-2}$. At the opposite end, Arcos and Bobal had a density of 209 and 205 stomata mm$^{-2}$, respectively (Table 5).

**Table 5.** Anatomical and morphological characteristics of stomatal and epidermal cells from Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta leaves.

| Variety | SD | SL | ED | ECA | SI |
|---|---|---|---|---|---|
| Arcos | 209 a | 22.7 a | 3785 c | 265 b | 6.5 abc |
| Bobal | 205 a | 25.0 c | 2695 a | 372 d | 7.5 cd |
| Forcallat | 280 d | 23.2 a | 3434 b | 295 c | 7.3 bc |
| Garnacha | 236 bc | 26.0 d | 2626 a | 382 d | 8.5 d |
| Monastrell | 257 cd | 24.6 bc | 4280 d | 234 a | 6.2 ab |
| Veremeta | 226 ab | 24.2 b | 4119 cd | 244 ab | 6.0 a |

SD: stomatal density (stomata mm$^{-2}$); SL: stomatal length (μm); ED: epidermal density (epidermic cell mm$^{-2}$); ED: epidermal density (epidermic cell mm$^{-2}$); ECA: epidermal cell area (μm$^2$); SI: stomatal index. Mean values within a column separated by different lowercase letters are different ($p < 0.05$).

Stomata also differed in length among varieties; Arcos and Forcallat showed the shortest stomata, with an average length of 22.7 and 23.2 μm, respectively, while Garnacha showed the longest length (26.0 μm) (Figure 3). A higher percentage of small stomata, with a length < 22 μm, were observed in Arcos and Forcallat, whereas the highest percentage of long stomata, >27 μm, was noted in Garnacha and Monastrell. As for the epidermic cells, a lower number of cells that were larger in size were counted in Garnacha and Bobal (Table 5). A higher epidermal density was recorded in Monastrell and Veremeta. They both had the lowest epidermal cell area and stomatal index. Regarding stomatal traits, Monastrell showed a higher stomatal density than Veremeta. Finally, the highest stomatal index was noted for Garnacha.

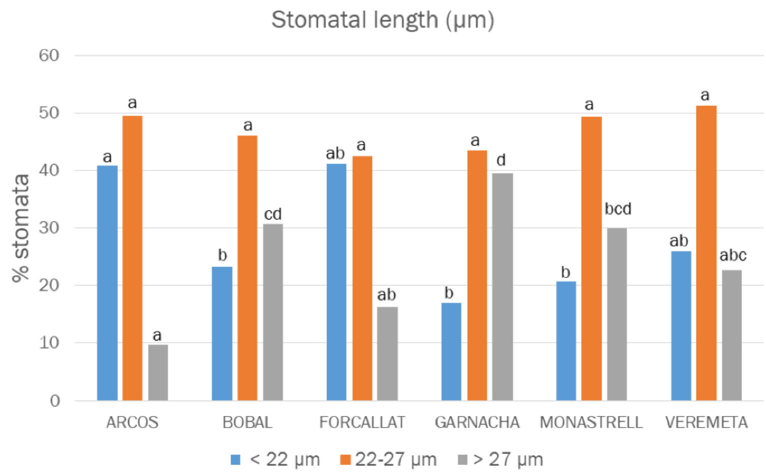


**Figure 3.** Stomata frequency (%) with lengths < 22μm, between 22–27 μm, and >27 μm in leaves from grapevine varieties. Mean values within a column separated by different lowercase letters are different ($p < 0.05$).

## 4. Discussion

Obtaining knowledge about traditional varieties and exploring intravarietal variability is of interest to avoid genetic erosion and to select varieties that could be adapted to climate change, which includes an increase in drought periods as consequence of erratic precipitation patterns and high temperature, and negative changes in fruit quality, fundamentally by a decoupling between technological and phenolic maturity [1,21]. To deal with this, researchers are investigating several adaptation strategies, which include the selection of drought-resistant varieties or clones, to understand the differences in drought tolerance among existing grapevine varieties [3,7,8] and to delay berry ripening [22]. In this work, we analyzed ampelographic and agronomic traits as well as physiological and anatomical features related with drought resilience in five traditional Mediterranean varieties (Arcos, Bobal, Forcallat, Garnacha, and Monastrell) and a variant of the variety Monastrell (Veremeta clara). All these varieties were present in the area of study prior to phylloxera arrival [23]. Bobal, Garnacha, and Monastrell have economic importance [20,24] and have been described in [25] as being drought tolerant. Currently, they occupy 152.829 ha of Spanish vineyards [26]. Garnacha (Grenache) is spread worldwide and Monastrell is grown in some areas of France, where it is known as Mourvedre [20]. Regarding the minor varieties Arcos and Forcallat, no information is available for their drought tolerance, and no ampelographic description for the variety Arcos, which is sometimes confused with Forcallat, has been given [27]. As both are late-ripening varieties, their recovery can be of interest in the context of climate change. In addition, there has been an increased interest in minor varieties recently to diversify wine production.

Ampelographic characterization is the first step needed to characterize plants that have not been previously described as well as to determine the specific features of the plants under study, as variability is commonly found in old varieties such as those we analyzed. As a result of our characterization, Arcos, whose SSR profile and chlorotype we reported in [27], has been described for the first time. Recently, Arcos has been included as a synonym for the variety Fumat in the VIVC (Vitis International Variety Catalogue) database, but any information for ampelographic traits have been added. Regarding the name Fumat, a similar name 'Fuma/es' has been used in some places of Valencia (Spain) for the variety Merseguera [28]. Ampelographic characterizations have also provided useful knowledge for differentiating this minor variety from Forcallat, both sometimes mistaken by farmers [27]. In fact, their bunches and grapes were very similar and both shared traits such as late ripening and growth characteristics (semi- upright or upright) (Table 1). Between the Veremeta clara and Monastrell varieties, great variability (for leaves, bunches and berries traits, vigor, and ripening) has been found despite both sharing the same SSR profile as we expected due to Veremeta being considered as one of the synonyms for this variety [29]. Regarding the adjective 'clara' (light), this is related with the leaf color, as it was corroborated with the colorimetric assay of the leaves, and also with the loose bunch of Veremeta, as this name is also used to indicate this characteristic in the area where it is grown. Compact bunches are more susceptible to diverse pests and diseases such as Botrytis bunch rot and *Lobesia boltrana*, which cause serious economic damage in grapevines [30–32]. Therefore, the loose bunches of Veremeta clara could be of interest in minimizing damages and reducing the chemicals needed for their control. Differences in vigor and grape composition were also observed among the analyzed varieties. However, an exhaustive characterization will be performed in future along the ripening period, which differs among them, to determine the evolution in sugar and pH levels, organic acids, anthocyanin, and color.

The variety Monastrell, which was autochthonous and the most representative of Alicante province, is well adapted to dry conditions and it has been described, along with with Bobal and Garnacha, as drought tolerant [25]. However, Garnacha has also been reported as having a very low tolerance to water stress [33], which could indicate there is genetic variability among the evaluated accessions as we found when analyzing several accessions of these varieties (data not shown). The evaluation of the stomatal conductance, and other related parameters, in the plants of these three varieties and others from Forcallat, Arcos, and Veremeta has reported information about their behavior under rain-fed conditions and in a semi-arid climate under a rainfall regime ranging from 430 to 506 mm/years. Stomatal conductance is a key trait in the regulation of the whole carbon and water balance and represents an integrative parameter that reflects the water stress experienced by plants [10]. Although all the varieties under study can be considered adapted to these conditions where they have been growing for more than 45 years, differences in gs among them were found. In addition, differences for gs were observed between measuring devices. As reported in a meta-analysis made in [33] and in a specific comparison carried out by [34], higher gs values were recorded when using porometers with respect to infra-red gas analyzers. Despite this, similar differential behavior among the varieties was observed independently of the measuring device; Arcos and Forcallat maintained lower gs rates along the culture with respect to the rest of the varieties and could be classified as pessimistic or isohydric varieties in our environmental conditions [35,36], whereas the rest showed high gs values along their cultures. On the dates when Ψstem was measured and based on [37], the plants were under moderate ($-0.8 > \Psi$stem $> -1.2$) to severe drought stress (Ψstem $< -1.2$), reaching values of Ψstem $< -1.6$ MPa. Considering this parameter, not all the cultivars and plants reached the same stress level at the same moment as reported by [38]. This may be a consequence of soil or rootstock–scion interactions or differences in the canopy (vigor and leaf area). The most stressed varieties in our field were Arcos and Forcallat. Nevertheless, both showed the highest WUEi (ratio between leaf net photosynthesis and stomatal conductance), which is considered a good parameter for selecting water-saving varieties [6,7]. Despite the differences for Ψstem, all the varieties regulated the stomata-reducing gs when Ψstem increased (Figure S5) and increased WUEi at the end of the culture period by reducing gs (from 20 August to 10 September; Table 4); Monastrell and Bobal were the most efficient after Arcos and Forcallat. Grapevine genotypes with a propensity for reduced gs can perform better in low-water-availability conditions [39]. Several strategies to improve this trait in grapevines have been proposed [7]. In addition, intravarietal grapevine variability in WUEi has been reported in some grapevine varieties such as Tempranillo [40] with the possibility of selecting more efficient clones. In our work, intravarietal variability for Monastrell was also found, which showed a higher WUEi than the variant Veremeta clara. The WUEi values obtained for Monastrell agreed with those reported by [41] in plants of this variety at a similar Ψstem ($-1.4$ MPa). Our results for Garnacha also agreed with those reported by [38], who found a non-water saving behavior in this variety. In relation to other traits that can be implicated in gs regulation, it was recently reported in tomato introgression lines derived from *Solanum pennellii* that higher trichome densities resulted in improved WUE, especially under water-deficit conditions [42]. In our work, an abundance of trichomes were found in Arcos and Forcallat and practically no trichomes were observed in Garnacha (Figure S3). Other differences among the varieties for stomatal conductance sensitivity could be related to the vulnerability of xylem cavitation and/or in the perception of abscisic acid, as well as other interconnecting chemical and hydraulic signals in which aquaporins could be involved [43–45]. Some studies have shown that ABA increases only when gs falls to levels lower than 50 mmol $m^{-2}$ $s^{-1}$, suggesting that early stomatal closure, as in Arcos and Forcallat, is not ABA driven, although it is difficult to absolutely discard this due to interconnections of several biochemical and hydraulic parameters [3].

The analysis of stomata characteristics that also influence stomatal conductance showed differences among the varieties as has been reported in other V. vinifera cultivars [11,46,47]. The range for stomatal density that we noted was even larger than that reported by [46] (50–400) and the averaged values inside the shortest ranges reported by [47] (170–250). Similarly, a wider range for stomata length was found in our work as compared to that reported by [48] (8.3 to 47.3 µm). Arcos and Forcallat, both showing the lowest gs rates throughout the growing period, and the highest WUEi, coincided in having a greater number of small stomata. Between these varieties, the total stomata densities were higher in Forcallat. Leaf stomatal density and gs have not been correlated in other works [49,50]. Despite this, a high stomatal density coupled with lower stomatal dimensions are features that minimize transpiration and are considered adaptations of the cultivars to water stress [12]. Smaller stomata are thought to open and close faster as compared to larger stomata while using less energy, and thus they can open under conditions where larger stomata stay closed [51,52]. This could explain the greatest differences in gs values between the measurement made in the am and pm that were found in Arcos and particularly in Forcallat on 10 August 2020.

## 5. Conclusions

As a result of this work, we have described for the first time the ampelographic traits for the minor variety Arcos and enriched the limited knowledge about another minor variety, Forcallat, which it is sometimes mistaken for. The analysis of stomatal conductance and other related traits have indicated that, under our growing conditions, both varieties Forcallat and Arcos had isohydric and water-saving behavior. These varieties also presented the highest densities of small stomata and trichomes, which are traits related to water stress adaptation. In addition, late-ripening character is interesting in the context of climate change for improving wine composition, particularly in warm growing areas. Indeed, Forcallat and Arcos are also of interest in the context of wine diversification. The features and knowledge about the physiological, anatomical, and agronomical behavior of Bobal, Garnacha, Monastrell, and Veremeta under rain-fed regime were also reported in this work. In addition, a great variability was obtained when comparing Monastrell and Veremeta clara, which shared the same SSR profile. Although all the compared varieties can be considered to be adapted to semi-arid conditions, the differences observed among them, especially between Monastrell and Veremeta, make this germplasm interesting for future studies to deepen our understanding of the mechanisms underlying their resilience to semi-arid climates.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092234/s1, Figure S1: Meteorological data in the area of study (by month and year); Figure S2: Growth of Arcos, Forcallat, Garnacha, and Bobal. Details of leaves from Arcos and Forcallat; Figure S3: Upper and lower side of leaves from Arcos, Forcallat, Bobal, Garnacha, Monastrell, and Veremeta; Figure S4: Images of bunches from the grapevine varieties Arcos, Bobal, Garnacha, Forcallat, Monastrell, and Veremeta on 10 August 2021; Figure S5: Correlation of stomatal conductance (gs) and stem water potential (Ψstem) of grapevine varieties Arcos, Bobal, Garnacha, Forcallat, Monastrell, and Veremeta on 20 August 2021; Table S1: Annual meteorological data in the area of study; Table S2: Molecular profile of the grapevine varieties Arcos, Bobal, Forcallat, Garnacha, Monastrell, and Veremeta clara analyzed by the following 15 SSRs markers title; Table S3: Cielab color parameters for leaves of Arcos, Bobal, Forcallat, Garnacha, and Veremeta.

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