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Identification and Characterization of Relict Olive Varieties (*Olea europaea* L.) in the Northwest of the Iberian Peninsula

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Abstract: Olives (*Olea europaea* L.) are an important crop in the Mediterranean Basin, but it is not well-known that they have also been grown in other areas, such as Galicia in northwestern Spain. Although commercial production ended long ago in this peripheral growing region, it remains home to olive resources that are well-adapted to the prevailing environmental conditions, providing a valuable but largely undocumented source of genetic variation. Following a survey of Galicia to locate examples of centuries-old olive trees, those detected were subjected to molecular characterization using a set of microsatellite markers, as well as full botanical characterization using the features established by the International Union for the Protection of New Varieties of Plants, along with others proposed by the present authors. These procedures allowed 11 undescribed varieties to be identified, which are new genetic resources that might be of use in olive improvement programs or studies of how the species adapts to different climates. The trees also underwent preliminary health checks, allowing disease-free specimens of each variety to be propagated. The addition of this material to the Community Plant Variety Office's register of commercial varieties is underway.

Keywords: centuries-old specimens; conservation; genetic resources; microsatellites; botanical characters; genetic diversity; health status

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1. Introduction

Olive trees (*Olea europaea* L.) have a long, productive life and can survive in adverse conditions [1]. The valorization of historic agricultural landscapes and the protection of germplasm resources from genetic erosion is considered a priority by the international community. The Mediterranean Basin is very rich in olive germplasm [2]. Although Galicia in northwestern Spain lies outside the area in which olive trees are commonly grown, they have been cultivated in this region for centuries, as evidenced by its many oil presses [3], the remains of olive seeds at archaeological sites [4], and historic references [5,6].

Galicia, the climate of which is influenced by the Atlantic Ocean, is a refuge of agricultural biodiversity, both for woody [7,8] and herbaceous crops [9–11]. Historically, Galicia has been an area of small farms, often with difficult terrain in which mechanization has been hard to implant; indeed, some work is still performed (on a small scale) by hand. Natural factors have not defined the historical presence or absence of olive cultivation in this region. Its distribution, in fact, has been a consequence of political and administrative decisions that explain its limited presence in this area of the Iberian Peninsula until today. For example, several authors [6,12,13] have indicated that the olive orchards in Galicia have been abandoned since the time of Philip IV due to the tribute imposed on each olive tree by order of his prime minister the Count-Duke of Olivares (1621–1643). From then on, 70% of the olive oil has been imported from Andalusia and the rest from the neighboring country of Portugal [6].

In the following centuries and up to the present day, improvement in the production of olive oil in the large oil-producing centers of southern Spain continued to discourage

its cultivation in the Levant and northeastern Iberian Peninsula. Nevertheless, in certain areas of Galicia, olive groves continued to form part of the traditional agricultural landscape, although often as a marginal crop due to their location on steep slopes or poorly developed soils. This adaptation to different pedoclimatic contexts has favored the emergence of new genotypes or heterogeneous regional populations that are highly different at the morphological, molecular, and agronomic levels [14].

In his work "Viaje a Galicia" (A Journey to Galicia), which was written in 1745 [15], Sarmiento wrote that many olive trees once existed in the Province of Pontevedra, but that by about 1740, only a few isolated specimens were left "like ornamentation for a Palm Sunday procession". The same author, in his major work entitled "De historia natural y de todo género de erudición. Obra de 660 pliegos" (Natural History and all Kinds of Erudition, a Work of 660 Pages) [5], devotes several pages of Tome 1 (of five) to "oil in Galicia", recording that olive trees grew well in all parts, "from Padron to—and including all—the Bishopric of Tuy, Quiroga and Valdeorras, and in nearly all the Bishopric of Orense, where the land is very good for olives. […] Best of all, as though not to be inferior to Galicia's other crops, the trees bear great quantities of olives". His words are echoed by other authors, such as [6,16]. Nowadays there is no such abundance; although the old olive orchard was maintained until a few decades ago, these too were eventually abandoned. However, some of the region's old, local varieties still exist, represented by large, centuries-old trees, usually either isolated in gardens or near churches (given the symbolic value of olives trees in Christianity), or growing in the mixed woodland that eventually took over their orchards. Recent years have seen a number of articles on Galicia's peripheral olive production and the varieties grown [17–19]. Indeed, our group undertook an exhaustive survey in search of these ancient trees [17,20].

The last decade has seen interest surge in the recovery of this biodiversity, as well as in the development of new agricultural alternatives linked to olive cultivation in Galicia—interests now shared by local, regional, and national authorities. Somewhat akin to wine, unique types of olive oil are also becoming of more interest to consumers. There are now many local growers ready to cultivate "native" olive trees on land that is currently abandoned and covered in brush and weeds. This "on farm" conversion will no doubt help prevent the disappearance of this exclusive biodiversity and, by providing jobs, perhaps help tackle the loss of population from rural areas.

The wet, mild climate of Galicia is very different to that of the rest of the world's olive growing areas, and the region's varieties appear to be well-adapted to it. They could, therefore, provide material that might be used in breeding programs or in studies of how olive trees adapt to different climates. The fact that (until very recently) no olive material has been brought into Galicia for several centuries only increases the scientific interest in its native varieties.

Molecular markers, especially microsatellites (SSR), have been successfully used to identify monumental, ancient native or locally cultivated olive trees throughout the Mediterranean Basin in Algeria [21,22], Montenegro [23,24], Italy [14,25,26], Greece [27,28], Turkey [29,30], the Maltese Islands [31], and Spain [1]. These markers have also proven to be very suitable for germplasm collection management [2,32–34]. For this, SSRs can quickly provide a preliminary identification of an olive variety [1,33,35,36] and have been proved to be very effective in identifying and discriminating olive varieties (always complemented by botanical and agronomic description) thanks to their transferability, high variability, and codominance. Although significant efforts have been made to align a range of SSR data to allow comparison among standardized databases, SSRs are yet to become official markers of olive identity (unlike for grapevine [37]). Neither does the use of these markers alone completely identify or characterize a variety. They do, however, reliably provide a means of identifying candidate varieties that can then be described botanically (this requires the collection of data over several growing cycles, but the results are legally recognized) [38].

Phytosanitary checks are a further required for the conservation of olive germplasm [39]. Olive trees are propagated vegetatively, but the material used should never be compromised by pathogens [39,40]. Obtaining healthy germplasm is an important goal; germplasm provided by Galicia's ancient trees therefore needs to be checked.

The aim of the present work was to reveal the existence of unexplored genotypes in northwestern Spain, which are locally grown in remote sites or are of minor commercial interest but of high value for biodiversity conservation and breeding, to completely described these relict olive varieties, and to undertake a preliminary examination of their health status. All these are essential steps prior to the use of this rediscovered plant material in new breeding programs or to its certification (production of true to type and pathogen-free plants) and commercial exploitation.

2. Materials and Methods

2.1. Plant Material

A survey of Galicia had previously located vestiges of old olive production represented by centuries-old trees [17]. Figure 1 shows the areas surveyed, some of which are mentioned as producing olives in the old literature. In each visited area, local people were interviewed to collect information on the existence of ancient olive trees, along with possible local names of varieties, the agronomic characteristics of these varieties, the use of the oil produced, and pertinent local history, legends, and ethnographic data, etc.

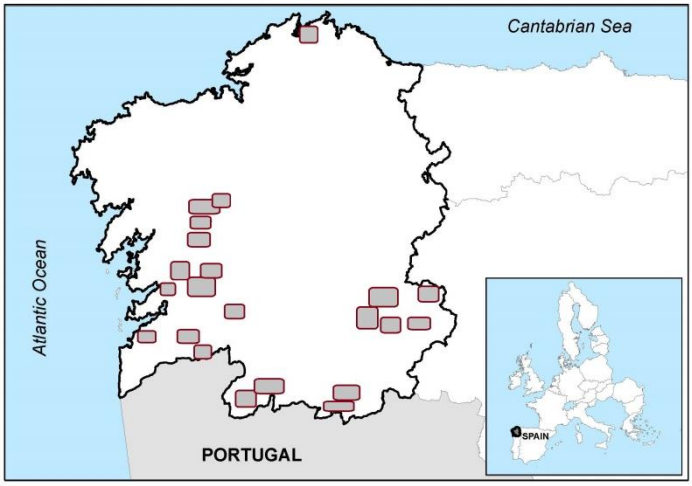

Figure 1. Areas surveyed in Galicia (shaded rectangles) in the search for ancient olive trees.

A total of 117 ancient trees were selected in this study, which met the following requirements: to be clearly centuries-old, as manifested by the size of their trunks and the references made to them by different generations of the owning families. Some of the centuries-old trees detected were no longer used for an agricultural purpose, although a number of these retired trees had taken on an ornamental or other role. The ancient trees selected were photographed, and their GPS data were recorded. To protect them from rapidly growing commercial interests, they were not marked in any way, nor will their exact locations be made known. Some specimens of "Arbequina"—a Spanish variety very recently brought to Galicia (highlighting the growing interest in olive production)—were marked to later act as controls.

2.2. DNA Extraction and Microsatellite Analysis

Young leaves were taken from branches of the present year's growth in the crown. All were stored at −80 °C until use. Total DNA was extracted from approximately 20 mg of finely ground powder of the young leaves combining the CTAB method [41] with the use of the Maxwell® PureFood Extraction Kit (Promega, Madison, WI, USA) and a

Maxwell® 16 MDx robot. The quantity and quality of the extracted DNA were examined using a NanoDrop® ND1000 spectrophotometer (Waltham, MA, USA). This DNA was then characterized using 15 SSR markers (Tables 1 and S1) [42–44]. The SSR regions were amplified, and PCR reactions were performed in a final volume of 20 µL, with 50 ng of template DNA, 1X PCR buffer (Biotools, Madrid, Spain), 200 µM of individual dNTPs (Roche, Germany), 0.3 units of Taq DNA polymerase (Biotools, Madrid, Spain), and 0.3 µM of each primer. Forward primers were labeled with one of the four fluorescent dyes, 6FAM™ (DCA11, GAPU-71B, UDO99-011, and UDO99-019), VIC® (DCA09, UDO99-024, and UDO99-043), NED™ (DCA03, DCA15, GAPU-59, and GAPU-101), and PET® (DCA05, DCA14, DCA18, and GAPU-103-A). The reaction conditions were: denaturing at 94 °C for 5 min, 35 cycles at 94 °C for 20 s, annealing at 50/53/55 °C (optimized for each SSR) for 30 s, 72 °C for 30 s, and an extension step at 72 °C for 8 min followed by conservation at 4 °C. Amplification products were verified using 3% agar gel electrophoresis using 5 µL of each PCR product and an NZYDNA Ladder V® size marker (Nzytech, Lisbon, Portugal) before separation using an ABI PRISM® 3100 device (Applied Biosystems, Waltham, MA, USA) and employing a GeneScan-400HD [ROX]® (Thermo Fisher, Waltham, MA, USA) size marker. Fragment size was determined using Geneious R.11 software (https://www.geneious.com (accessed on 15th May 2023)) [45]. The "Arbequina" control material was treated in the same way to facilitate comparisons with database entries/results of other authors. SSR profiles were compared to those described elsewhere.

Additionally, for each SSR marker, the number of alleles per locus (Na), effective number of alleles (Ne), Shannon information index (I), observed (Ho) and expected heterozygosity (He), and fixation index (F) (Table 2), were calculated using GeneAlEx ver. 6 as a plugin module within Microsoft Excel [46]. Subsequently, a genetic similarity dendrogram was constructed using similarity's simple matching coefficient and the agglomerative unweighted pair group method with arithmetic mean (UPGMA) algorithm.

Table 1. Microsatellite profiles, varietal names assigned, and number of individual trees with the same SSR profile (N).

		SSRs LOCI						
Name Given	**N**	**ssrOeUA-DCA03**	**ssrOeUA-DCA05**	**ssrOeUA-DCA09**	**ssrOeUA-DCA11**	**ssrOeUA-DCA14**	**ssrOeUA-DCA15**	**ssrOeUA-DCA18**
Brava Gallega	53	237–251	207–207	184–194	140–179	190–190	243–254	171–181
Brétema	28	228–251	201–207	172–184	130–161	173–180	243–254	171–181
Carapucho	3	237–243	207–207	182–206	140–140	190–190	254–254	171–187
Carmeliña	2	243–247	207–207	162–184	140–179	190–190	254–263	173–177
Folgueira	11	243–247	207–207	162–206	161–179	180–190	263–263	173–181
Hedreira	1	237–251	207–207	162–208	161–179	180–190	243–263	173–177
Mansa Gallega	13	228–243	201–207	182–184	130–140	173–190	254–254	171–187
Maruxiña	1	237–251	207–207	162–184	179–179	190–190	243–254	173–181
Susiña	1	237–247	207–207	162–184	140–179	190–190	254–263	179–181
Xoana	3	241–247	195–207	172–194	146–161	178–190	243–263	173–181
Santiagueira	1	243–251	207–207	184–194	179–179	190–190	243–263	173–181
Arbequina (Control)	3	230–241	203–207	184–206	140–179	190–190	243–263	169–179

		SSRs LOCI							
Name given	**N**	**GAPU-59**	**GAPU-71B**	**GAPU-101**	**GAPU-103-A**	**UDO99-011**	**UDO99-019**	**UDO99-024**	**UDO99-043**
Brava Gallega	53	212–222	127–141	192–218	138–138	114–127	130–130	166–186	174–206

Brétema	28	212–222	124–141	190–192	165–165	110–112	130–130	178–186	172–214
Carapucho	3	212–222	124–141	190–218	138–165	112–114	100–130	178–186	172–218
Carmeliña	2	212–222	127–141	198–218	138–153	114–127	130–130	166–186	210–214
Folgueira	11	212–222	127–141	192–218	189–189	122–127	130–130	186–186	174–218
Hedreira	1	212–212	121–141	198–218	189–189	112–114	130–130	186–186	174–218
Mansa Gallega	13	222–222	124–127	190–192	165–165	112–127	100–130	166–178	172–216
Maruxiña	1	212–222	127–141	198–218	138–153	112–114	130–130	186–186	174–204
Susiña	1	222–222	141–141	192–192	138–138	122–127	130–130	166–186	174–206
Xoana	3	212–212	127–141	198–218	177–189	120–122	130–130	186–186	174–176
Santiagueira	1	212–222	127–141	198–200	189–189	114–127	130–130	186–186	210–218
Arbequina (Control)	3	222–222	121–141	184–206	153–162	112–124	130–155	202–202	176–176

Table 2. Size range (base pairs), number of different alleles (Na), number of effective alleles (Ne), information index (I), and observed (Ho) and expected (He) at each SSR locus for the olive varieties analyzed.

SSR Locus	Size Range	Na	Ne	I	Ho	He
ssrOeUA-DCA03	228–251	7	5.633	1.809	1.000	0.822
ssrOeUA-DCA05	195–207	4	1.380	0.589	0.308	0.275
ssrOeUA-DCA09	162–208	8	5.045	1.828	1.000	0.802
ssrOeUA-DCA11	130–179	6	3.634	1.466	0.769	0.725
ssrOeUA-DCA14	173–190	4	1.633	0.774	0.385	0.388
ssrOeUA-DCA15	243–263	3	2.965	1.093	0.769	0.663
ssrOeUA-DCA18	169–187	7	5.281	1.778	1.000	0.811
GAPU-59	212–222	2	1.988	0.690	0.615	0.497
GAPU-71B	118–141	5	3.045	1.291	0.923	0.672
GAPU-101	184–218	7	4.507	1.658	0.923	0.778
GAPU-103-A	138–189	6	4.072	1.537	0.385	0.754
UDO99-011	110–127	7	4.630	1.670	1.000	0.784
UDO99-019	100–155	3	1.266	0.431	0.231	0.210
UDO99-024	166–202	4	2.126	1.012	0.462	0.530
UDO99-043	172–218	9	7.191	2.071	0.923	0.861
All loci	100–263	65	5.633	1.809	1.000	0.822
Mean		5.467	3.626	1.313	0.713	0.638

2.3. Botanical Characterization

Botanical characterization was performed for those plants with different SSR profiles. This was undertaken following the criteria of the International Union for the Protection of New Varieties of Plants (UPOV)—specifically those in the UPOV norm "Protocol for distinctness, uniformity and Stability test for Olea europaea L. (UPOV code: OLEAA_EUR" adopted 28/11/2012 and the International Olive Council) [47,48]. The latter UPOV protocol describes the methodology to follow to meet the demands of the European norm Nº2100/94 regarding the "Community Plant Variety Rights" proposed by the Community Plant Variety Office (CPVO). For these characterizations, 40 mature leaves were taken from the central area of growing, one-year-old branches. The leaf characters proposed by Rallo et al. [48] and The International Olive Council (IOC) were recorded (Table 3). In addition, "average leaves" for each candidate variety were constructed using previously reported methods [17]. This was achieved using the same leaves as examined in the botanical characterization process. Briefly, each of the 40 leaves was photographed, and the

lengths and angles shown in Figure 2 were recorded with the help of ImageJ 1.5.3 software [49].

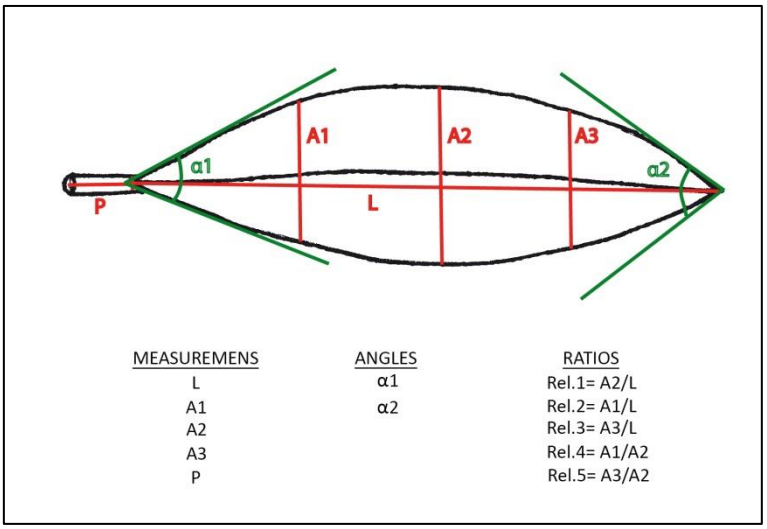

Figure 2. Lengths and angles measured in the determination of the "average olive leaves".

Foliar morphologies were compared statistically [17] after calculating the ratios Rel.1 = A2/L; Rel.2 = A1/L; Rel.3 = A3/L; Rel.4 = A1/A2; and Rel.5 = A3/A2 (Table 4). This method does not, therefore, contemplate absolute leaf size, which can depend on soil and climatic conditions, etc. Principal component analysis (PCA) was then performed to group varieties by leaf similarity. This was performed using XLSTAT 2023.3.1 (Addinsoft, New York, NY, USA) software. PCA biplots were also prepared using XLSTAT 2023.3.1.

Table 3. Leaf qualitative botanical characteristics (mode values according to the corresponding UPOV scale).

	Leaf Descriptors			
	UPOV 5 **Length**	**UPOV 6** **Width**	**UPOV 7** **Ratio Length/Width**	**UPOV 9** **Curvature of Longitudinal Axis**
Brava Gallega	5 Medium	3 Narrow	7 Very elongated	2 Straight
Brétema	5 Medium	5 Medium	3–5 Slightly-Moderately elongated	2 Straight
Carapucho	5 Medium	3 Narrow	5 Moderately elongated	2 Straight
Carmeliña	5 Medium	3 Narrow	7 Very elongated	2 Straight
Folgueira	5 Medium	3 Narrow	7 Very elongated	2 Straight
Hedreira	5 Medium	5 Medium	5 Moderately elongated	2 Straight
Mansa Gallega	5 Medium	5 Medium	5 Moderately elongated	2 Straight
Maruxiña	5 Medium	3 Narrow	7 Very elongated	2 Straight
Susiña	5 Medium	3 Narrow	5 Moderately elongated	2 Straight
Santiagueira	5 Medium	3 Narrow	7 Very elongated	2 Straight
Xoana	5 Medium	3 Narrow	7 Very elongated	2 Straight
Arbequina	3 Short	5 Medium	3 Slightly elongated	3 Recurved

Table 4. Ratios (see Figure 2) calculated from the measured leaf angles and lengths (M = mean, SD = standard deviation, CV = coefficient of variance).

	REL.1 = A2/L			**REL.2 = A1/L**			**REL.3 = A3/L**			**REL.4 = A1/A2**			**REL.5 = A3/A2**		
	M	**SD**	**CV**	**M**	**SD**	**CV**	**M**	**SD**	**CV**	**M**	**SD**	**CV**	**M**	**SD**	**CV**
Brava Gallega	0.18	0.02	0.12	0.12	0.02	0.14	0.14	0.02	0.11	1.54	0.18	0.12	1.23	0.15	0.12
Brétema	0.23	0.03	0.15	0.24	0.09	0.38	0.23	0.05	0.21	1.12	0.44	0.39	1.04	0.26	0.25
Carapucho	0.15	0.03	0.17	0.19	0.04	0.19	0.16	0.03	0.19	0.81	0.06	0.08	0.85	0.06	0.08
Carmeliña	0.18	0.02	0.09	0.12	0.01	0.12	0.15	0.01	0.09	1.50	0.14	0.09	1.24	0.17	0.14
Folgueira	0.16	0.02	0.15	0.10	0.02	0.16	0.13	0.02	0.16	1.52	0.18	0.12	1.27	0.19	0.15
Hedreira	0.15	0.03	0.17	0.20	0.03	0.17	0.16	0.03	0.21	0.77	0.07	0.09	0.83	0.10	0.12
Mansa Gallega	0.14	0.03	0.25	0.20	0.04	0.19	0.17	0.04	0.23	0.70	0.09	0.13	0.84	0.08	0.10
Maruxiña	0.17	0.02	0.12	0.13	0.02	0.15	0.14	0.02	0.15	1.32	0.12	0.09	1.09	0.15	0.14
Santiagueira	0.23	0.02	0.11	0.17	0.02	0.12	0.17	0.02	0.12	1.33	0.13	0.10	1.01	0.13	0.13
Susiña	0.18	0.02	0.14	0.13	0.02	0.16	0.14	0.02	0.15	1.43	0.12	0.08	1.14	0.14	0.12
Xoana	0.16	0.03	0.16	0.12	0.02	0.17	0.13	0.02	0.19	1.26	0.13	0.10	1.06	0.18	0.17
Arbequina	0.17	0.04	0.22	0.23	0.04	0.17	0.18	0.03	0.18	0.14	0.18	0.12	1.08	0.18	0.16

All trees included in the analysis could produce fruit, but some, although still productive, were abandoned and had no fruit production. For those trees that produced fruit, forty ripe drupes were also collected from each tree for botanical characterization (which includes recording drupe weight and size and taking different measurements, etc.) using UPOV criteria (UPOV Code: OLEAA_EUR). Once this was completed, all endocarp material was removed, cleaned using 50% sodium hypochlorite in water, and dried in an oven at 35 °C until a constant weight was reached to finally examine botanically and morphologically. The botanical characterization of the drupes was repeated over several years to determine whether the characters recorded remained stable over time (important for reliably distinguishing between varieties). To group varieties by drupe and endocarp similarity, a scatter plot was constructed from drupe/endocarp length and drupe/endocarp width ratios, calculated for each variety.

To determine the relatedness between olive genotypes based on drupe endocarp descriptive characteristics, the squared Euclidean dissimilarity index was employed. Subsequently, hierarchical cluster analysis was performed using the unweighted pair group method with arithmetic average (UPGMA) clustering algorithm, while a dissimilarity dendrogram was constructed using the XLSTAT software package.

2.4. Physicochemical Characterization of the Drupes and of the Oil Obtained from Them

During the 2020 harvest, olives were taken (when possible) from the different trees for analysis using the ABENCOR® (Sevilla, Spain) method [50]. This analysis provided preliminary information regarding the chemical composition and organoleptic qualities of the olives and the oil obtained from them. Olives were also collected from the "Arbequina" control trees. For the oils, the water and volatile compound content, total fat content (TFC), and fat content per dry weight of olives (FDW) (used to detect ripeness (optimum 43–45%)) were determined. In some cases, the oil from different trees of the same molecular and botanical characteristics was mixed to have sufficient material for testing. The varieties assigned the names "Susiña" and "Santiagueira" did not produce enough olives in any year for the above analyses to be performed.

The physicochemical properties (free acidity, peroxide index, absorbance of UVA light at K 270, K 232, and Delta-K, water content, and impurities) that determine oil quality, according to regulation EU 2568/91 and its amendments (European Commission 1991 and 2007) and the IOC, were then determined (IOC/T.20/Doc.N°15/Rev.7/2015). In addition, the water and volatile compound and ether-insoluble impurity contents were determined according to the latter authority's criteria (COI/T.15/NC nº 3/Rev. 10).

2.5. Plant Health

Each of the varieties confirmed by SSR analysis and botanical characterization were examined to determine their status regarding the pathogens contemplated by EU regulation 2016/2031:

- Fungi: Verticillium dahliae (a regulated, nonquarantinable disease (RNQD))
- Bacteria: Xylella fastidiosa (a priority quarantinable disease (QD)) and Pseudomonas savastanoi pv. Savastanoi (RNQD)
- Viruses: Arabis mosaic virus (ArMVoo), cherry leaf roll virus (CLRVoo), strawberry latent ring spot virus (SLRSVo), and cucumber mosaic virus (CMVoo).

All checks were performed at an external laboratory officially recognized for the detection, according to EPPO protocols, of viruses, viroids, bacteria, fungi, and phytoplasmas cataloged as reportable/quarantinable in the European Union. Viruses and bacteria were sought through the extraction of their nucleic acids from the plant material. For the diagnosis of Verticillium dahliae, samples were first incubated at 26 °C in potato dextrose broth for 72 h. DNA was then extracted from anything growing in the broth. Pathogen species were identified by amplifying their DNA using appropriate PCR methods. All

analyses (performed on several samples of each plant material) were performed in duplicate.

3. Results

3.1. SSR Analyses

Table 1 shows the SSR profiles detected for the 117 samples of plant material and the number of plants for each profile, along with the varietal name assigned. The profile of the "Arbequina" controls is also shown.

The 117 trees analyzed with 15 SSRs corresponded with 11 genotypes (Table 1). A total number of sixty-five different alleles were detected (Table 2), of which DCA09 and UDO99-43 loci carried the highest number, with eight and nine alleles, respectively, and GAPU-59 was the least polymorphic as it showed only two alleles (Table 2). The number of effective alleles ranged from 1.266 (UDO99-019) to 7.191 (UDO99-043), with a mean value of 3.626. On average, the expected heterozygosity (He) was lower than the observed (Ho), although three loci (DCA-14, GAPU-103, and UDO99-24) showed an opposite trend (Table 2). All olive varieties were successfully identified using 15 SSR markers (Figure 3).

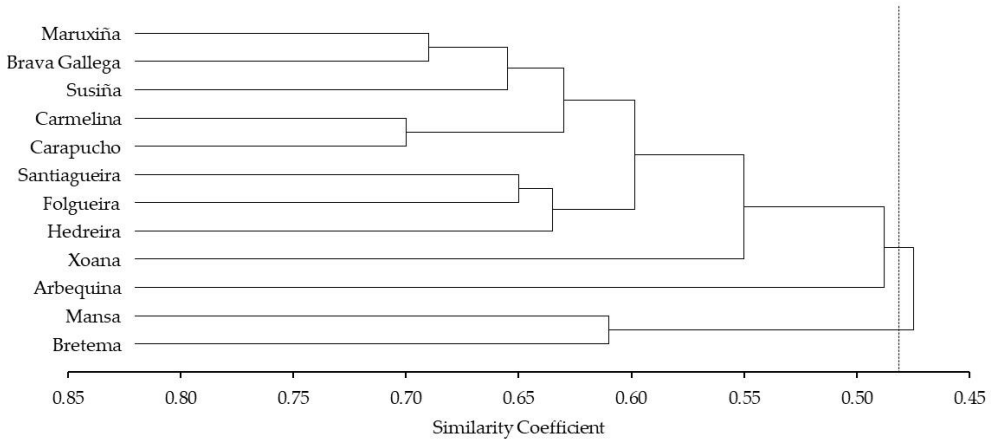

Figure 3. UPGMA dendrogram of studied olive trees, including "Arbequina" as reference cultivar, based on SSR markers.

3.2. Botanical Characterization Results

Table 3 shows the mode values for the leaf UPOV characteristics. Table 4 shows the values of the ratios calculated using the different leaf lengths and angles. Table 5 and Figure 4 show the results of the PCA performed with the same ratios. The first two axes (Prin 1 and 2) accounted for 92.68% of the variance, and the first three accounted for 98.26% (Table 5). With respect to Prin 1, the variable with most positive weight was the Rel2 ratio, which relates the width of the leaf blade's zone near the peduncle to the leaf length. The variables with the most negative weight were Rel.4 and Rel.5 ratios, which reflect the relationship between leaf widths taken at different points. With respect to Prin2, the variable with greatest positive weight was Rel.1, which relates the width of the leaf at its central section to the total length of the leaf. In Figure 4, for Prin 1 and Prin2, the varieties separate with respect to the morphology of their leaves; half of the varieties group toward the left, with lanceolate leaves. The variety "Brétema", however, is placed toward the upper right of the graph; its leaves are markedly elliptical, with the blade wider at the base near the peduncle. The variety "Santiagueira" had elliptical leaves that were homogeneous in width along most of their length. Finally, "Carapucho", "Hedreira", and "Mansa Gallega" grouped together because their leaves were not very wide at the base.

Table 5. Value, proportion, and percentage of accumulated variance obtained in PCA using leaf length and angle ratios.

	PCA Variable		
Component	**Autovalue**	**Proportion**	**Acc. Var.**
1	2.89	0.5771	0.5771
2	1.75	0.3497	0.9268
3	0.28	0.0559	0.9826
4	0.09	0.0173	0.9999
5	0.00	0.0001	1.0000

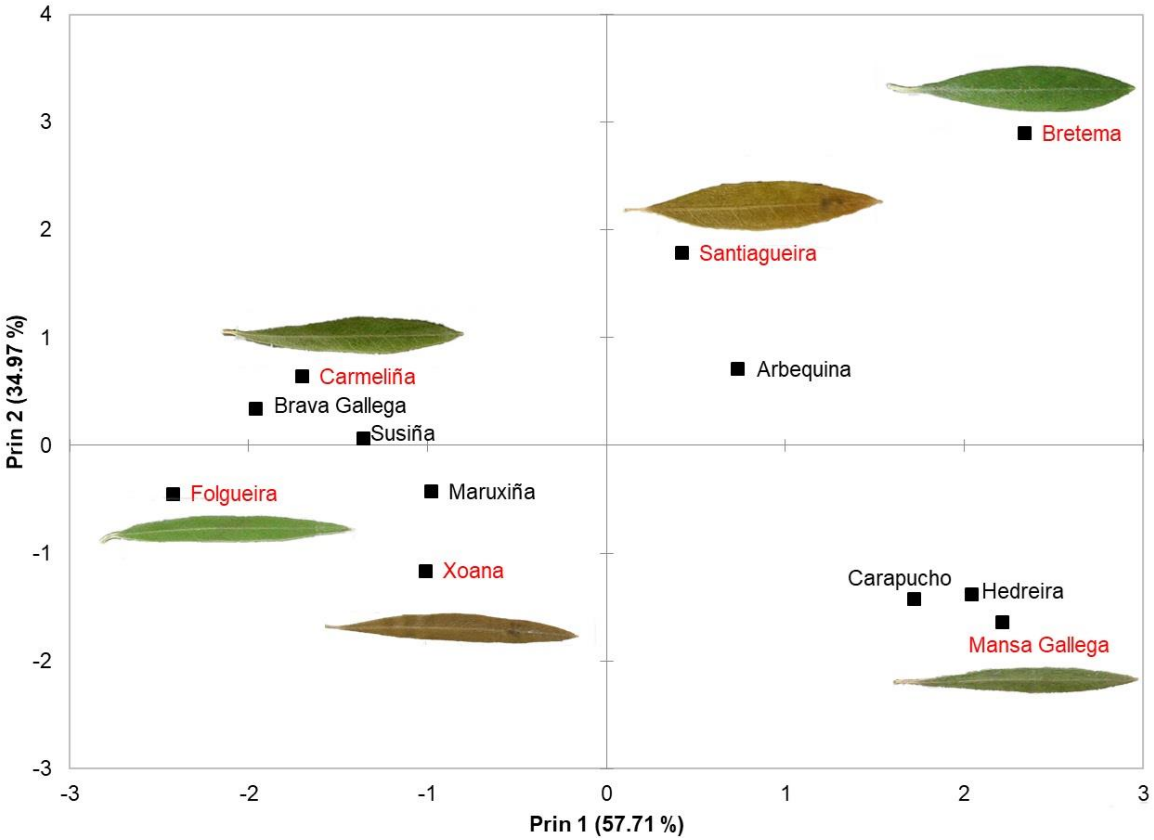

Figure 4. Results of PCA (Prin 1 and 2) performed using the determined leaf angles and lengths and distribution of varieties with respect to leaf morphology. Leaves are not represented to scale.

Tables 6–8 show the qualitative and quantitative results for the drupes and endocarps. The proportion of the drupe occupied by the endocarp for each of the varieties for which it has been possible to take measurements of their fruits is shown in Figure 5.

Table 6. Drupe qualitative botanical/morphological characteristics (mode values (according to the corresponding UPOV scale)).

	Drupe Descriptors *							
	UPOV16	**UPOV18**	**UPOV22**	**UPOV23**	**DiamMaxDrup**	**UPOV24**	**UPOV25**	**UPOV26**
Brava Gallega	5	5	3	2	2	3	1	3
	Medium	Moderately elongated	Black	Weakly asymmetric	Center	Rounded	Absent	Truncate
Brétema	5	5	3	2	2	3	1	3
	Medium	Moderately elongated	Black	Weakly asymmetric	Center	Rounded	Absent	Truncate
Carapucho	5	7	3	2	2	3	1	3
	Medium	Very elongated	Black	Weakly asymmetric	Center	Rounded	Absent	Truncate
Carmeliña	5	5	2	2	2	3	1	3
	Medium	Moderately elongated	Dark violet	Weakly asymmetric	Center	Rounded	Absent	Truncate
Folgueira	5	5	3	2	2	3	1	1
	Medium	Moderately elongated	Black	Weakly asymmetric	Center	Rounded	Absent	Rounded
Hedreira	5	5	2	3	1	2	2	3
	Medium	Moderately elongated	Dark violet	Strongly asymmetric	Toward the base	Obtuse	Moderate	Truncate
Mansa Gallega	3	5	3	2	2	3	1	3
	Low	Moderately elongated	Black	Weakly asymmetric	Center	Rounded	Absent	Truncate
Maruxiña	5	5	2	2	2	3	3	1
	Medium	Moderately elongated	Dark violet	Weakly asymmetric	Center	Rounded	Strong	Rounded
Susiña	3	3	1	1	2	3	1	3
	Low	Slightly elongated	Medium violet	Symmetric	Center	Rounded	Absent	Truncate
Xoana	7	5	1	3	2	2	2	3
	High	Moderately elongated	Medium violet	Strongly asymmetric	Center	Obtuse	Moderate	Truncate
Arbequina	3	3	3	1	1	3	1	3
	Low	Slightly elongated	Black	Symmetric	Toward the base	Rounded	Absent	Truncate

* UPOV16: weight; UPOV18: ratio length/width in position A; UPOV22: skin color at ripeness; UPOV23: symmetry at position A; DiamMaxDrup: maximum diameter; UPOV24, shape of apex at position A; UPOV25: nipple; UPOV26: shape of base at position A. No data are shown for variety "Santiagueira" with no production during the analyzed years.

Table 7. Endocarp qualitative botanical/morphological characteristics (mode values (according to the corresponding UPOV scale)).

	Endocarp Descriptors *										
	UPOV31	**UPOV32**	**UPOV33**	**UPOV34**	**UPOV35**	**UPOV36**	**UPOV37**	**UPOV38**	**UPOV39**	**UPOV40**	**DMax Endo**
Brava Gallega	2	5	2	1	2	1	3	9	2	2	2
	Moderately elongated	Medium	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Present	Rounded	Medium	Centered
Brétema	2	5	3	1	1	3	3	9	2	2	2
	Moderately elongated	Medium	Strongly asymmetric	Symmetric	Less than 7	Strongly grouped	Rounded	Present	Rounded	Medium	Centered
Carapucho	3	5	2	1	2	1	1	9	1	2	2
	Very elongated	Medium	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Acute	Present	Acute	Medium	Centered
Carmeliña	2	7	2	1	2	1	3	9	1	2	2
	Moderately elongated	High	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Present	Acute	Medium	Centered
Folgueira	2	5	2	1	2	1	3	9	1	2	3
	Moderately elongated	Medium	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Present	Acute	Medium	Toward the apex
Hedreira	2	7	2	1	2	1	3	9	2	2	1
	Moderately elongated	High	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Present	Rounded	Medium	Toward the base
Mansa Gallega	2	3	2	1	2	1	3	9	2	1	2
	Moderately elongated	Low	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Present	Rounded	Weak	Centered
Maruxiña	2	7	2	1	2	1	1	9	3	2	2
	Moderately elongated	High	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Acute	Present	Truncate	Medium	Centered
Susiña	u	3	2	1	2	1	3	9	2	1	2
	Moderately elongated	Low	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Present	Rounded	Weak	Centered
Xoana	2	7	2	1	2	1	1	9	1	3	2
	Moderately elongated	High	Weakly asymmetric	Symmetric	Between 7 and 10	Evenly distributed	Acute	Present	Acute	Strong	Centered
Arbequina	1	3	1	1	2	1	3	1	2	2	2
	Slightly elongated	Low	Symmetric	Symmetric	Between 7 and 10	Evenly distributed	Rounded	Absent	Rounded	medium	Centered

* UPOV31: ratio length/width; UPOV32: weight; UPOV33: symmetry at position A; UPOV34: symmetry in position B; UPOV35: number of grooves on basal end; UPOV36: distribution of grooves on basal end; UPOV37: shape of apex at position A; UPOV38: mucron; UPOV39: shape of base at position A; UPOV40: surface roughness; DMax Endo: maximum diameter.

Table 8. Mean weight (g), length (mm), width (mm), and width/length ratio of drupes and endocarps.

		Drupes			Endocarps		
Variable	**Variety**	**Mean**	**SD**	**CV (%)**	**Mean**	**SD**	**CV (%)**
Weight (g)	Brava Gallega	3.16	0.92	29.16	0.43	0.08	19.07
	Brétema	2.01	0.56	27.96	0.38	0.09	23.98
	Carapucho	2.94	0.71	24.16	0.35	0.06	18.07
	Carmeliña	2.17	0.48	22.25	0.47	0.06	13.6
	Folgueira	2.84	1.00	35.36	0.39	0.08	21.67
	Hedreira	2.70	0.31	11.51	0.49	0.06	12.97
	Mansa Gallega	1.03	0.20	19.8	0.23	0.04	18.34
	Maruxiña	2.41	0.62	25.83	0.60	0.09	15.56
	Susiña	1.66	0.32	19.28	0.26	0.06	20.92
	Xoana	4.16	0.84	20.27	0.51	0.09	16.78
	Arbequina	0.77	0.11	14.65	0.26	0.03	11.55
Length (mm)	Brava Gallega	21.08	2.21	10.47	14.93	1.69	11.3
	Brétema	19.18	2.02	10.51	14.09	1.50	10.64
	Carapucho	21.62	2.02	9.34	15.69	1.82	11.58
	Carmeliña	18.88	1.47	7.77	14.10	0.93	6.58
	Folgueira	20.01	2.53	12.65	14.34	1.44	10.01
	Hedreira	19.72	1.11	5.63	13.08	1.09	8.3
	Mansa Gallega	15.18	1.08	7.09	11.24	0.87	7.76
	Maruxiña	19.96	1.76	8.83	15.11	0.97	6.38
	Susiña	14.70	0.92	6.27	9.84	1.00	10.12
	Xoana	23.95	2.12	8.85	16.27	1.75	10.76
	Arbequina	12.00	0.60	5	9.92	0.81	8.2
Width (mm)	Brava Gallega	15.53	1.75	11.27	7.42	0.59	7.91
	Brétema	13.33	1.45	10.86	7.55	0.67	8.87
	Carapucho	14.77	1.49	10.06	6.53	0.41	6.32
	Carmeliña	13.70	1.20	8.73	7.70	0.52	6.8
	Folgueira	15.04	2.09	13.89	7.19	0.61	8.5
	Hedreira	15.42	0.69	4.47	7.82	0.27	3.5
	Mansa Gallega	10.53	0.74	7.06	6.19	0.49	7.83
	Maruxiña	14.14	1.59	11.22	8.57	0.75	8.76
	Susiña	13.31	1.00	7.47	6.91	0.48	6.97
	Xoana	17.80	1.58	8.85	7.83	0.58	7.4
	Arbequina	9.87	0.82	8.32	6.64	0.35	5.22
Width/Length ratio	Brava Gallega	0.74	0.07	8.98	0.50	0.10	18.86
	Brétema	0.70	0.06	8.66	0.53	0.05	9.41
	Carapucho	0.69	0.09	12.44	0.42	0.04	10.30
	Carmeliña	0.73	0.05	7.08	0.55	0.04	7.97
	Folgueira	0.75	0.05	7.12	0.50	0.04	8.43
	Hedreira	0.78	0.05	5.96	0.60	0.06	9.29
	Mansa Gallega	0.70	0.05	6.64	0.55	0.04	8.04
	Maruxiña	0.71	0.04	5.11	0.57	0.04	6.75
	Susiña	0.91	0.06	8.05	0.71	0.08	11.26
	Xoana	0.75	0.06	7.87	0.49	0.07	14.13
	Arbequina	0.82	0.06	7.26	0.67	0.07	10.60

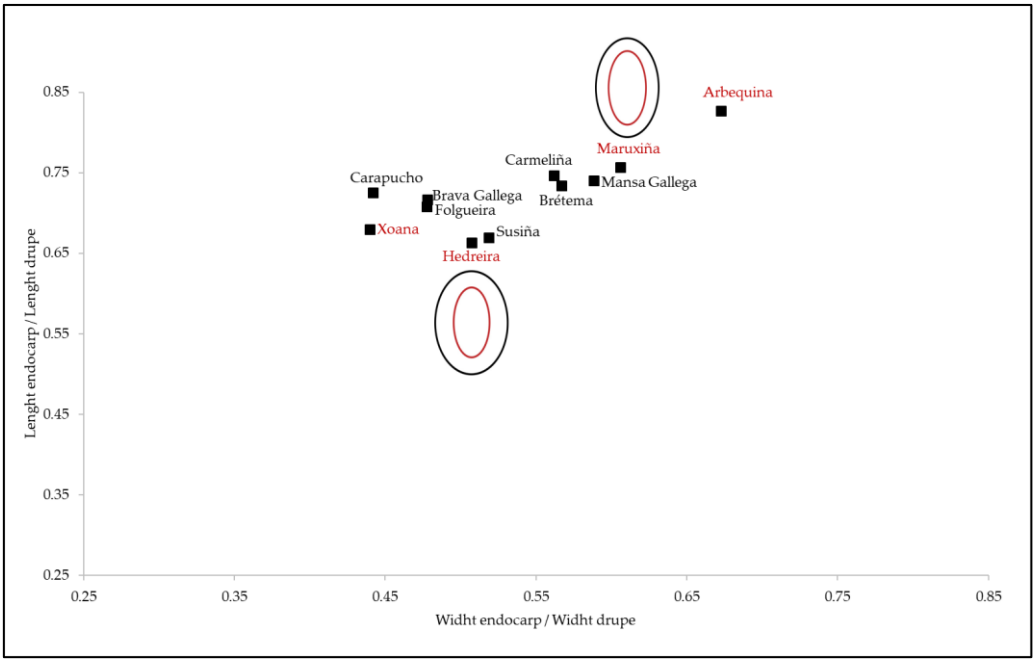

Figure 5. Scatter plot constructed from endocarp/drupe length and endocarp/drupe width ratios.

Regarding the qualitative parameters of drupes and endocarps, the clusters resulting from the UPGMA analysis (Figure 6), four groups have been defined. Two of them include a single variety ("Arbequina" and "Brétema"), one group is composed of two varieties ("Hedreira" and "Xoana"), and a fourth group is made up of the remaining varieties included in the characterization. However, even for this large group, the parameters used are adequate to successfully differentiate all the varieties studied. The control variety used, "Arbequina", which does not have its origin in the study area, is completely separate from the rest of the native varieties in terms of the characteristics of its fruits and endocarps. The autochthonous variety "Brétema" also separates itself from the rest of the varieties, showing several characteristics in its endocarps that are rare among the rest of the examined endocarps, such as very asymmetrical endocarps or those with few grooves and grouped together. The varieties "Hedreira" and "Xoana" form a fourth group that is differentiated from the rest by certain characteristics mainly related to the apex of the drupe.

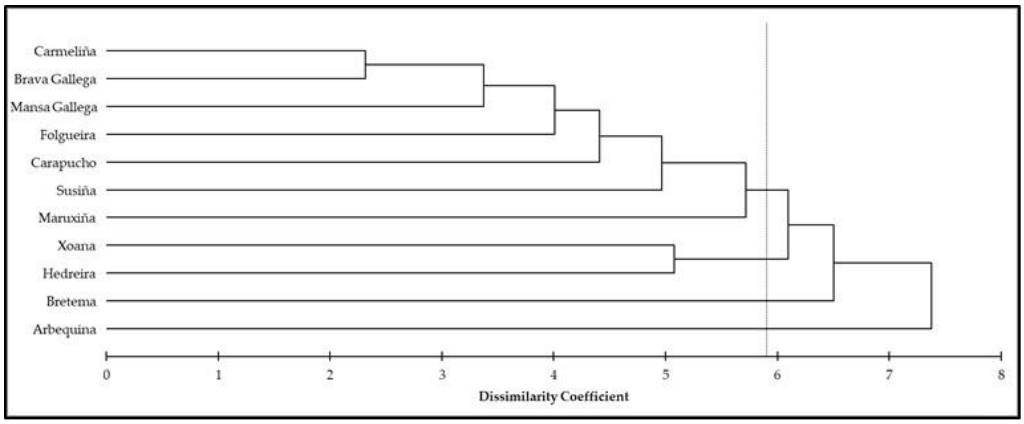

Figure 6. UPGMA dissimilarity dendrogram analysis using 19 traits for drupe and endocarp of the studied trees, including reference cultivar "Arbequina", based on the Euclidian distance and unweighted pair-group average agglomeration method. The variety "Santiagueira" did not produce olives during the study period and could not be included in this analysis.

Figures 7 and 8 show representative images of typical leaves, drupes, and endocarps for each variety.

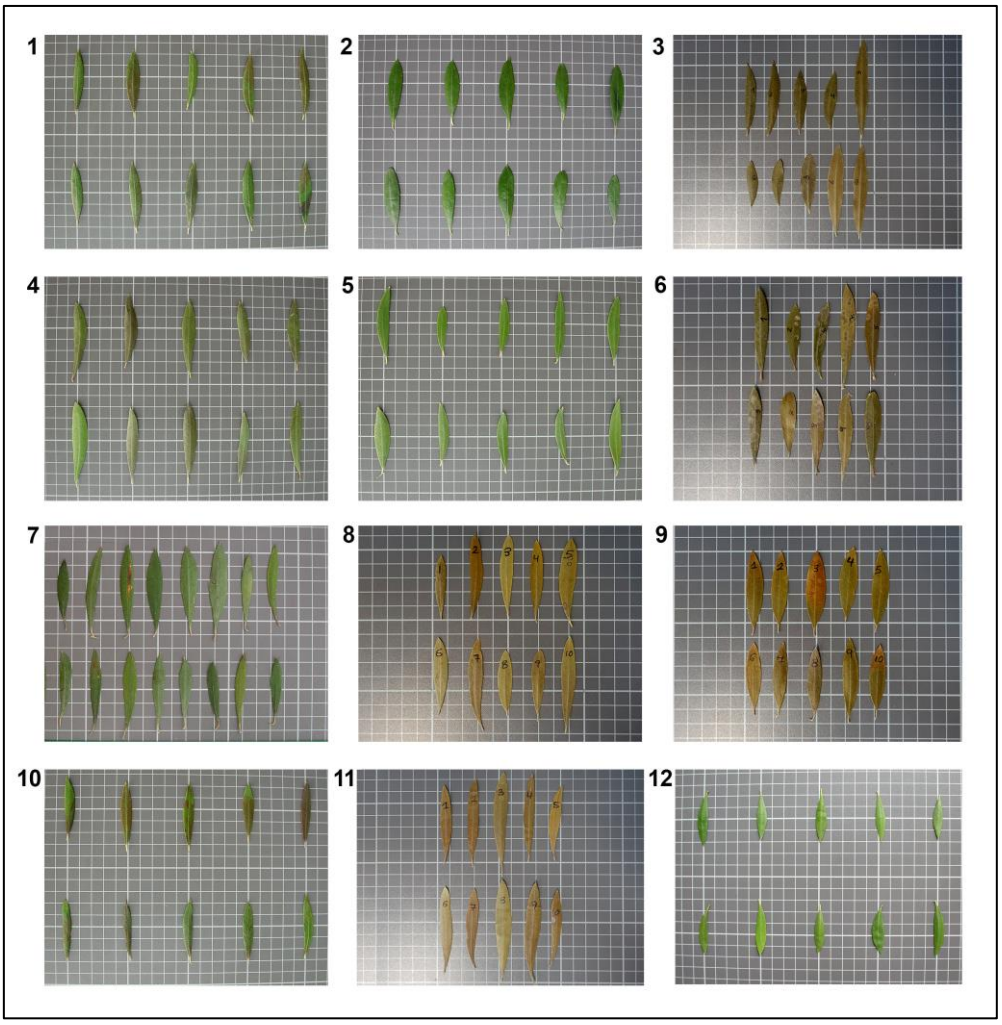

Figure 7. Pressed leaves of the studied varieties. 1—"Brava Gallega", 2—"Brétema"; 3—"Carapucho"; 4—"Carmeliña"; 5—"Folgueira"; 6—"Hedreira"; 7—"Mansa Gallega"; 8—"Maruxiña"; 9—"Santiagueira"; 10—"Susiña"; 11—"Xoana"; 12—"Arbequina".

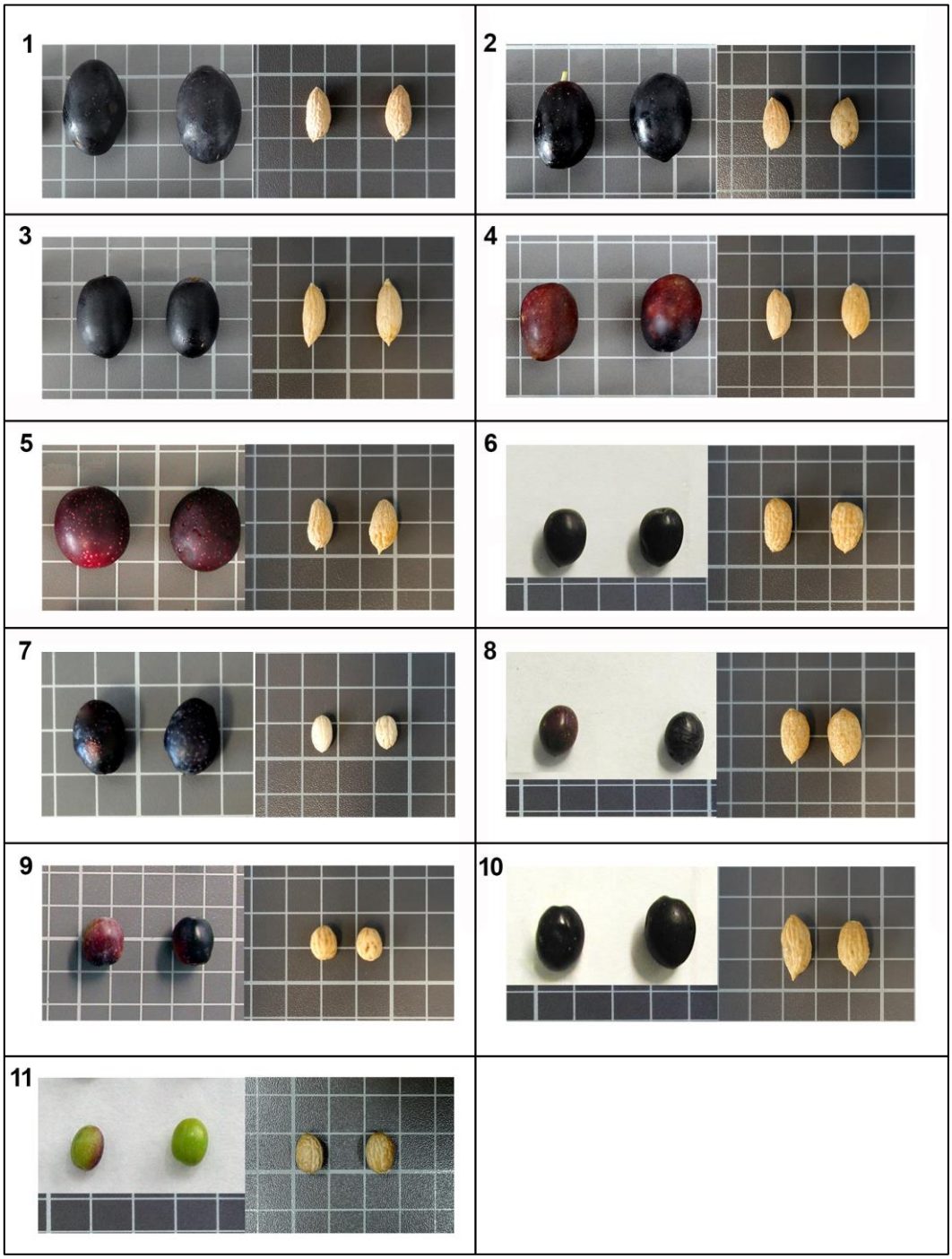

Figure 8. Drupes and endocarps of the studied varieties, harvested at the same time with different maturity degrees. 1—"Brava Gallega", 2—"Brétema"; 3—"Carapucho"; 4—"Carmeliña"; 5—"Folgueira"; 6—"Hedreira"; 7—"Mansa Gallega"; 8—"Maruxiña"; 9—"Susiña"; 10—"Xoana"; 11—"Arbequina".

3.3. ABENCOR® Variables

Figure 9 shows the results of the ABENCOR analysis of the different drupes. "Folgueira", and "Maruxiña" varieties presented the lowest water and volatile content (WVC) (41.16% and 44.33%, respectively), while those of the "Carapucho" variety presented a WVC content of 63.30, higher than 50%, which is the average value cited in the literature [47,48]. The total fat content (TFC) was less than the standard 25% in all the analyzed samples, ranging from 10.36% in "Carapucho" to 24.13% in "Folgueira". The fat content of the olive without considering the moisture content or fat per dry weight (FDW) was also

calculated, allowing for comparison between samples. The highest FDW was observed in the olives of "Xoana" (45. 58%), "Folgueira" (41.01%), and "Hedreira" (40.14%), while the olives of "Maruxiña", "Carapucho", and "Carmeliña" showed an FDW of under 30%.

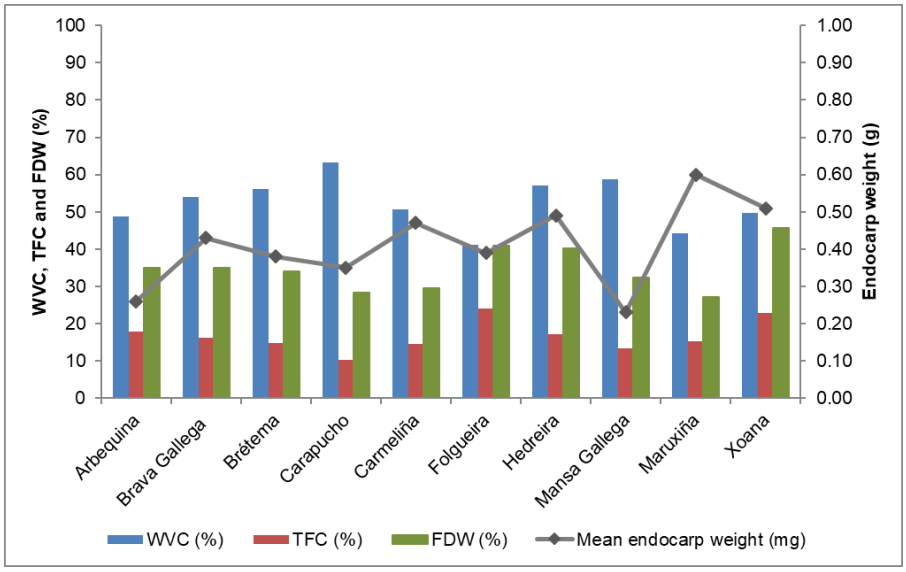

Figure 9. ABENCOR® analysis of drupes: results for 2020. The water and volatile compound content (WVC) was determined gravimetrically, total fat content (TFC) was determined using Soxhlet analysis, and the fat per dry weight (FDW) was determined as FDW = (TFC/(100 − WVC) × 100).

The olive oils extracted using the ABENCOR method were analyzed for the physicochemical parameters that determine the quality of olive oils according to the regulations of the European Union and the International Oil Council (IOC) (Table 9). For both the quality parameters "degree of free acidity" and "peroxide index" and those related to ultraviolet absorbance (K232, K270, and Δk), all of the analyzed samples met the threshold limits set by the legislation for extra virgin olive oil (EVOO). The water and volatile material content was higher than 0.2% in all samples, which is the limit set by the IOC for EVOO (IOC/T.15/NC N°3/Rev.13). All varieties showed a content of impurities insoluble in petroleum ether less than 0.1% (m/m), thus meeting the threshold established by the IOC for EVOO.

Table 9. Physicochemical properties of the oils produced in 2020 and analytical methods used.

	Free Acidity (% Oleic Acid)	**Water and Volatile Compound (% m/m)**	**Ether-Soluble Impurities (% m/m)**	**Peroxide Index (meq O_2 Peroxidized per kg Oil)**	**K 270 ***	**K 232 ****	**ΔK**
Variety	**Rule 2568/91 CEE Annex II**	**UNE 55 020**	**UNE 55 020**	**Rule 2568/91 CEE Annex III**	**Rule 2568/91 CEE Annex IX**	**Rule 2568/91 CEE Annex IX**	**Rule 2568/91 CEE Annex IX**
Brava Gallega	0.37	0.32	0.03	3.05	0.13	1.76	0.00
Brétema	0.47	0.29	0.02	6.00	0.13	1.53	0.00
Carapucho	0.18	MD	MD	MD	MD	MD	MD
Carmeliña	0.46	0.82	0.03	3.90	0.13	1.42	0.00
Folgueira	0.20	0.32	0.03	2.50	0.12	1.57	0.00
Hedreira	0.22	0.30	0.04	4.30	0.17	1.43	0.00
Mansa Gallega	0.23	0.24	0.03	8.45	0.11	1.20	0.00
Maruxiña	0.27	MD	MD	MD	MD	MD	MD
Xoana	0.33	0.30	0.03	3.70	0.18	1.60	0.00
Arbequina	0.29	0.54	0.03	2.45	0.09	1.28	0.00
EVOO reference [$]	≤0.80	≤0.2	≤0.1	≤20	≤0.22	≤2.5	≤0.01

[$] EVOO = extra virgin olive oil; * K270 = absorbance of UVA at 270 nm; ** K232 = absorbance of UVA at 232 nm; MD: missing data.

3.4. Health Status of the Examined Trees

No genetic material belonging to *V. dahliae*, *P. savastanoi* pv. *Savastanoi*, X. *fastidiosa*, ArMV, CMV, CLRV, or SLRSV was detected in any plant material.

4. Discussion

This work describes a number of relict olive varieties native to Galicia (northwestern Spain). The trees representing them were located after exhaustive searches across the region. All were found in agricultural areas influenced by the Atlantic Ocean (with some Mediterranean features), far away from those parts of Spain where olives have been cultivated without interruption for centuries. The available historical information [16] makes it clear that Galicia was once a very productive olive growing area. In the mid-18th century, the Valdeorras, Quiroga, and Monterrei valleys were responsible for 80% of Galicia's olive oil production [6]. Olive growing only disappeared because of political decisions and the economic interests of figures in authority [6,20]; the recovery of the region's orchards should, therefore, be possible because the discovered trees are adapted to the prevailing environmental conditions. Future work should, however, explore the possible impact of climate change.

Little new olive material has been introduced into Galicia, leaving its native olive biodiversity intact. The very recent introduction of the varieties "Arbequina" and Picual has had no effect on the purity and uniqueness of the centuries-old trees detected. Our group possesses the only germplasm bank that conserves specimens of these newly identified varieties, but representative samples will be sent to The Worldwide Olive Germplasm Bank of Córdoba (WOGBC), Spain, where they can also be curated.

Over the last decade, the agricultural sector of northwestern Spain has shown growing interest in the recovery of olive production, with a particular focus on the use of regional varieties. The latter, however, requires that they first be formally identified. The only two such varieties recognized to date are "Brava Gallega" and "Mansa Gallega" [17]. Certainly, the existence of unnamed accessions has led to confusion and misidentifications. Properly identifying Galicia's native olive varieties is a vital step toward their official recognition (and indeed a requirement for their cultivation under current legislation) and the appropriate labelling of the oil they produce.

At the molecular level, 11 distinct genotypes have been differentiated within the 117 centenary olive trees studied, representing great variability (about 10%). The SSR profile most commonly detected among the examined trees was that of "Brava Gallega" (45.33%). This material was also classified as such using botanical analysis, confirming this variety to be the most common across the area surveyed. Some 13% of the trees were found to belong to the variety "Mansa Gallega". Some 28% and 11% belonged to the newly denominated "Brétema" and "Folgueira" varieties, respectively. The remaining profiles were represented by just 1–3 trees each. All the SSR profiles obtained were checked against those held in databases/reported in the literature [1,2,18,19,23,29,30,33,47,51–53]; those for the varieties "Brava Gallega", "Mansa Gallega", and the newly denominated "Folgueira" were detected.

In a previous preliminary work [17], profiles for the "Brava Gallega" and "Mansa Gallega" varieties were published using a similar set of microsatellite markers. In this previous work, only one specimen of the "Mansa Gallega" variety and two specimens of the "Brava Gallega" variety had been included. The profiles shown in the present work are the results of the analysis of a larger number of specimens of both varieties and present some minor adjustments made for the size of some alleles for some of the SSRs markers used. Furthermore, in relation to this preliminary work, the profiles noted as Unknown 1, 2, 3, and 5 were not found to correspond with any variety present at the WOGBC at that time or through comparison with databases and molecular profiles reported by other authors. These varieties are currently in the process of registration with the names shown in the present work as "Brétema" (formerly Unknown 1), "Carapucho" (Unknown 2),

"Hedreira" (Unknown 3), or "Folgueira" (Unknown 5), and the molecular profiles presented here also include minor adjustments in some loci compared to Gago et al. [17]. It is worth mentioning that the cultivar "Hedreira" was considered homozygous in the preliminary work for the DCA 15 locus as only one allele had been detected, but after repeating the analysis several times, a second allele for this locus was detected (Table 1).

The UPGMA dendrogram based on the SSR markers analyzed (Figure 3) placed the foreign variety "Arbequina", used here as a control or reference variety, in a single group (with a similarity coefficient of 0.48). For the remaining autochthonous varieties, the analysis has established different groupings. Further studies will be performed in the future to determine the possible relationships between these and other genotypes.

The profile for "Brava Gallega" was detected in two studies that characterized this germplasm[17,18]. The molecular profile and botanical description recognized by the CPVO for "Mansa Gallega" are those reported for this variety in the present work and not the material erroneously described by [18] or later by [19] as "Mansa" and "Mansa de Figueiredo", respectively. The molecular profile given for that material by the latter authors in fact corresponds to the variety here designated as "Folgueira", and both this name and the rigorous description of this variety provided in this work has been accepted by the CPVO and the official recognition process is nearing completion.

The most common leaf shape among the studied varieties was lanceolate (Figures 4 and 7). The "Brétema" variety is, therefore, easily distinguishable by its almost elliptical leaves. The range of leaf length was similar across all varieties, while the ratios Rel.1, Rel.2, and Rel.3 (which relate leaf width at different points to leaf length) showed more variability. The variety "Santiagueira" had lanceolate leaves which showed almost constant width along their length. In contrast, the leaves of "Carapucho", "Hedreira", and "Mansa Gallega" were narrower and pointier near the insertion of the peduncle. The remaining varieties had very similar leaves (quantitatively and qualitatively).

The drupes of the variety "Xoana" were the largest and heaviest, while "Arbequina", "Susiña", and "Mansa Gallega" had the smallest and lightest drupes and endocarps. The remaining varieties had drupes of intermediate size and weight (Table 8). Most varieties had drupes that were longer than they were wide (elongated in Table 6 or with the fewest width/length ratio in Table 8). Those of "Susiña" and "Arbequina", however, were more rounded. With the exception of the varieties "Carmeliña", "Hedreira", and "Maruxiña", drupe weight appeared to correlate with endocarp weight. For the three named varieties, the endocarp weight was heavy for the weight of the drupe. Drupe and endocarp shape also appeared to be related (especially for Carpucho, in which both were very elongated). The varieties "Susiña" and "Arbequina", however, had slightly elongated drupes but only slightly to moderately elongated endocarps (Tables 6–8).

According to the skin color of the drupe at ripeness (Table 6), the varieties "Carmeliña", "Hedreira", and "Maruxiña" produced dark violet drupes, while those of "Susiña" and "Xoana" had lighter shades of the same color. All the other varieties produced black drupes at ripeness. The skin color of the drupe observed in Figure 8 for some of the varieties did not match with the annotation in Table 6 for this parameter, as all the varieties represented in Figure 8 were harvested at the same time, independently of the maturity degree, while the description of the drupe color was conducted with olives at ripeness, as required by the UPOV code for this parameter.

The drupes of all varieties ranged from being weakly asymmetric to strongly asymmetrical, except for those of "Susiña" and "Arbequina", which always had small and symmetrical drupes, with a depression along the lateral suture in "Susiña" (Table 6 and Figure 8). The drupes of "Maruxiña" had a very evident nipple, while "Hedreira" drupes had a nipple of moderate size, and no nipple was present in "Xoana" drupes. "Hedreira" drupes had their maximum diameter toward the base, while in all other varieties, this was central. According to the shape of the base at position A, all the varieties had truncate drupes, except for "Folgueira" and "Maruxiña", in which the drupes were rounded for this parameter. In "Carapucho", "Maruxiña", and "Xoana", the endocarp apex was pointed,

while in the remaining varieties it was rounded. The surface of "Maruxiña" endocarps was rough with deep fibrovascular grooves that were somewhat grouped together near the lateral suture. "Xoana" endocarps were the roughest, while those of "Mansa Gallega" and "Susiña" were almost smooth. In the remaining varieties, the endocarp was of intermediate roughness. "Brétema" endocarps had very few fibrovascular grooves, which were grouped around the lateral suture (Figure 8). "Folgueira" and "Hedreira" were distinguishable by the maximum diameter of the endocarp appearing toward the apex in contrast to the central position occupied in the other varieties.

With respect to the ratio between endocarp and mesocarp represented in Figure 5, "Xoana" and "Carapucho" were separated with the lowest ratio (<0.45), while "Arbequina" was at the opposite extreme with a value higher than 0.65. This means that the former had a very high proportion of pulp, while "Arbequina" had the least amount of pulp. The rest of the varieties were located in intermediate positions.

The botanical cluster tree (Figure 6) constructed from 19 qualitative parameters of drupes and endocarps did not match the SSRs clustering. This is not surprising because these traits are rarely associate with the molecular ones. As expected, this analysis also showed "Arbequina", with a dissimilarity coefficient of 5.90, to be absolutely separated from the autochthonous varieties.

The results regarding the oil produced by these varieties, while preliminary, are sufficiently positive to suggest that experimental orchards should be established for more detailed work to be undertaken, comparing production and quality under similar edaphoclimatic and cultivation conditions. Some of the varieties showed good potential for the production of quality extra virgin olive oil (EVOO) and were sufficiently particular to stand out at market. The varieties "Folgueira" (41.34% FDW), "Hedreira" (40.14% FDW), and "Xoana" (45.58% FDW) produced oil well, while the other varieties did so more poorly. This might have been due to the growing conditions to which the representative trees were subjected (none received any care that might encourage oil production). In all other respects, the oils from all varieties had properties allowing their classification as EVOOs according to the IOC and EU Regulation 2568/91 (which establishes quality criteria) and its subsequent amendments.

Table 9 shows all the analyzed oils to have a water and volatile compound content of >0.2%, the upper limit set by the IOC for EVOOs and virgin olive oils (VOA). Following this criterion, all the present oils are classifiable as *lampant*, which is no doubt a consequence of the lack of care received by the trees. All the oils had <0.1% (m/m) ether-soluble impurities, which is the upper limit set by for EVOO and VOA by the aforementioned authority.

No pathogens were found infecting the trees, which is an important result with respect to their propagation. The practice of propagating olive trees via semi-woody cuttings has aided the spread of certain diseases, especially those caused by viruses. The absence of pathogens in the studied trees might be a consequence of their isolation and the lack of any import of olive material into Galicia until very recent times. Studies performed in other countries indicate different rates of infection for old olive orchards, which are as high as 87.6% in Apulia (Italy) [39] and 74.6% in Tunisia [54], down to 25% in Croatia [55] and 8.2% in Greece [56]. The holding in isolation of at least one pathogen-free specimen of each variety at our facilities opens the door to registration by the CPVO, later certification, and finally, transfer to nurseries and growers.

5. Conclusions

This work reveals the presence of previously unknown varieties of olive tree growing in Spain's northwest. This is an essential first step toward optimizing the preservation of the olive genetic resources and, consequently, for diversity and genetic studies. These varieties have interesting technological characteristics and deserve to be conserved and studied in depth. The rediscovered varieties have a very important value and can be exploited

by new breeding programs to produce new genotypes suitable for new conditions and emergent diseases and to obtain increasingly sustainable productions.

This new germplasm also has a direct commercial value. None of the trees examined showed any sign of disease requiring mandatory control measures, which should help in the registration of the varieties they represent. We have recently started the vegetative propagation using the cuttings of these genotypes for future agronomical characterization under the same soil and climatic conditions and to study their resistance levels to biotic and abiotic stresses.

Author Contributions: M.-C.M. was responsible for the acquisition of funding and project administration. M.-C.M., P.G., and J.-L.S. proposed this study, planned and directed it, set goals, undertook experimental work and analyses, interpreted the results, and wrote the draft of the manuscript. S.B. helped with statistical analyses and the writing of the original draft. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article and are available from the corresponding author [M.-C.M] on request.

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