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Study of Molecular Biodiversity and Population Structure of *Vitis vinifera* L. ssp. *vinifera* on the Volcanic Island of El Hierro (Canary Islands, Spain) by Using Microsatellite Markers

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Abstract: El Hierro island is postulated as the most biodiverse of the archipelago. To verify this hypothesis, the 87 individuals collected throughout the island were genotyped with 20 SSRs. As a result of this study, 28 varieties were described, 6 of which were new (Uval piñero, Uvalero volcánico, Pinar negro, Seis de Carlos, Tesoro blanco, Uval negro), and the first rose sport of the local Canary Islands variety Bermejuela was also found. Fifteen errors were detected in total. Eleven varieties were identified that were unknown to the vine growers and twenty individuals with variations (mutations) were found, of which two had already been described in a previous prospection in Lanzarote Island (intra-varietal variability). From this study, it is also proposed to incorporate 33 new names into the world database, corresponding mostly to the individuals and variations described for the first time, which represents a lexicographic enrichment. Finally, the singularity of the population of vines adapted to El Hierro island is demonstrated, not only with respect to the population of Canary Islands vines, but also with respect to the world population. The biodiversity and uniqueness of El Hierro and the Canary Archipelago reaffirm the proposal that the Canary Islands should be considered a world biodiversity centre.

Keywords: Vine (Vitis genre); SSR; characterisation; identification; volcanic; Canary Archipelago; El Hierro Island

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1. Introduction

Vitis vinifera L. is one of the oldest species in the Mediterranean basin, along with olive, wheat, and fig [1]. The European vine is a sarmentose vine belonging to the kingdom *Plantae*, division *Anthophyta* (*Magnoliophyta*), class *Magnoliopsida* (*Eudicotyledons*), subclass *Rosids*, order *Vitals*, family *Vitaceae*, subfamily *Viticoideae*, genus *Vitis*, and species *Vitis vinifera* L. according to the classical botanical classification [2]. Its genome is diploid with 19 chromosome pairs and an estimated size of 500 megabase pairs (Mbp) [1]. The species *Vitis vinifera* L. is divided into two subspecies, *Vitis vinifera* ssp. *sylvestris* (the wild form) and *Vitis vinifera* ssp. *vinifera* (the domesticated form). The wild vine is dioecious, with male and female species, whereas most modern cultivars (corresponding to the domesticated vine) are hermaphrodite plants. The vine is also highly heterozygous and requires vegetative propagation to maintain the differentiating characteristics of each variety [2].

Vitis vinifera spp. *vinifera* biodiversity originated at the end of the last glaciation of the Quaternary period (beginning of the Holocene) [3,4]. Wolkovich et al. [5] quantified

intervarietal variability in this species from its origins to the present day in more than 6000 cultivars. Also, these authors report that 12 varieties (Cabernet Sauvignon, Chardonnay, Merlot, Pinot noir, Syrah, Sauvignon blanc, Riesling, Muscat à petits grains blanc, Gewürztraminer, Viognier, Pinot blanc, and Pinot gris) represent between 70–90% of the world's current vineyard area [5]. This fact is causing an important homogenisation of commercial wines, which together with wine sector legislation (especially the Appellation d'Origine Contrôlée (AOC)), which limits the number of varieties to be cultivated) is causing an important vine genetic erosion [6]. Additional factors are responsible for vine genetic erosion, such as *phylloxera* plague (which almost devastated the entire world vineyard at the end of the 19th and beginning of the 20th century) and, nowadays, alongside the homogenisation of commercial wines, climate change. Twenty-first century climate change effects will lead to the disappearance or displacement of many current wine-growing regions, along with a change in grape cultivar distribution. Additionally, consumers of AOC bottled wines are beginning to grow tired of the well-known international varieties, like Cabernet Sauvignon, Merlot, and Chardonnay. In this sense, those wine consumers seek to experience new sensory perceptions (experiential marketing) [7]. Consequently, emerging market trends emphasise the search for a typical character in wines, which directly contributes to the recovery of varietal biodiversity. Furthermore, the conservation and study of grape varieties diversity will undoubtedly be one of the potential solutions to mitigate the effects of climate change [5,8].

The Canary Islands are part (together with the Azores, Cape Verde, Madeira, and the Ilhas Selvagens) of Macaronesia, a biogeographical zone formed by this group of five archipelagos that stretches from southwest Europe to northwest Africa (Figure 1). The Canary Islands archipelago, formed by eight main islands (Tenerife, Fuerteventura, Gran Canaria, Lanzarote, La Palma, La Gomera, El Hierro y La Graciosa) and five minor islands (Alegranza, Islote de Lobos, Montaña Clara, Roque del Oeste y Roque del Este), are part of the Spanish national territory and is located off the north-western part of the African continent [9].

Figure 1. Macaronesia map (**left**) [10] and Canary Islands archipelago (**right**) [11].

The first vine varieties cultivated on the Macaronesian islands were introduced by the Spanish and Portuguese [12]. One of the most relevant historical events in the wine sector is that *phylloxera* (which devastated European vineyards and caused a drastic reduction in local varieties) never attacked the Canary Islands but did affect the Azores and Madeira archipelagos. This has allowed the appearance of new phenotypes due to the accumulation of genetic mutations over five centuries to enable the adaptation of new phenotypes. Therefore, many of the varieties of *Vitis vinifera* L. in the Canary Islands are the result not only of natural selection and mutations but also of natural crosses and anthropogenic selection [13].

The present work focuses on the study of grapevine varieties on El Hierro island of (Figure 2). This island is the Canary Archipelago's westernmost and southernmost point and is situated between parallels 27″38′ and 27″51′ north latitude. El Hierro is a volcanic origin island, with an estimated geological age of 1.2 million years, being the youngest of the Canary Islands [9].

Figure 2. El Hierro. Satellite view obtained from the NASA World Wind programme [14].

The climatic variation in each area of El Hierro island is conditioned by orography, but it is the clouds and their moisture that play a determining role. The trade winds, together with the Canary Current (a cold Gulf Stream branching that separates in the Azores), mean that the island does not have as arid a climate as the Sahara, which is at the same latitude [9].

El Hierro has all the soil and climatic characteristics required for quality viticulture. It is typical of the island to grow vines on terraces in order to make the greatest possible use of soil in very small areas which are subject to heavy erosion. The 203 hectares of vineyards in El Hierro are distributed as follows: 50% are in the municipality of Frontera, occupying the north-facing slopes of the Valle del Golfo, and the rest are distributed between the municipalities of Valverde (47.5 hectares) and El Pinar (51.5 hectares). A total of 86% of the vineyard area is located between 200 and 400 metres above sea level [15]. As mentioned above, *Vitis vinifera* L. has evolved for more than 500 years in the Canary Islands, resulting in unique varieties that allow the archipelago to be classified as one of the world's main centres of vine biodiversity [16]. In terms of biodiversity, El Hierro island is the most biodiverse in the Canary Islands (so far), and the following local varieties have been identified: Burra volcánica (White (W)) [16], Verijadiego (W) [16–18], Verdello de El Hierro (W) [17,18], Huevo de gallo (W) [16,18], Mollar cano rosado (Rose (Rs)) [16], and Verijadiego negro (Black (B) [16]).

The main objective of this study is to characterise and identify unknown autochthonous grapevine varieties in order to preserve the biodiversity, both inter- and intra-varietal, and to increase the range of wines that can be marketed in the future in this island. At the same time, if necessary, any errors in the identification of varieties and lexicographical errors that may appear will be corrected, and, finally, a study of the population structure will be carried out to see the extent of the uniqueness of the population of El Hierro individuals.

2. Materials and Methods

2.1. Plant Material

Eighty-seven samples (grapevine shoots) of different *Vitis vinifera* L. individuals were collected in different areas of El Hierro island by means of a mass selection strategy carried out by the local winegrowers. It was estimated that the best time to collect the samples

would be during winter pruning (specifically, they were collected in January). On El Hierro, vineyards are planted with several varieties at the same time, following the Canary Islands traditional planting system. Once collected, grapevine shoots were stored at −20 °C until processing. Detailed information on the accessions analysed is shown in Table S1.

2.2. DNA Extraction and Purification

To extract the genetic material from the samples, the methodology proposed by Marsal et al. [19,20] was followed, with an adaptation based on the procedure of Fort et al. [21]. This method has been optimised by performing two chloroform washes, as this is more efficient in removing proteins. The quality of each extraction sample was evaluated with the help of the Thermo Fisher® Scientific NanoDrop TM 1000 Spectrophotometer (Waltham, MA, USA), which accurately measures the concentration and purity level of nucleic acids.

2.3. Simple Sequence Repeat (SSR) Markers

Grapevine samples were genotyped using 20 SSR markers, which were previously selected for their discrimination and polymorphism capacity based on previous studies: VVS2, VVS3, and VVS29 [22]; VVMD5, VVMD6, and VVMD7 [23]; VVMD27, VVMD28, and VVMD36 [24]; VrZAG21, VrZAG47, VrZAG62, VrZAG64, VrZAG79, and VrZAG83 [25]; SCU06vv [26]; VvUCH11, VvUCH12, and VvUCH19 [27]; VChr19a [28]. SSRs VrZAG47 and VVMD27 are not independent *loci*, meaning they amplify the same genome area. The difference between them is in the primer design [29]. Of all the SSRs, there are nine that the international scientific community [30,31] considers to be reference or international genetic markers: VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, and VrZAG79. This research was carried out with 7 international SSRs plus 13 non-international SSRs, which were chosen based on their characteristics (Table S2).

2.4. DNA Amplification and Polymerase Chain Reaction (PCR)

An Applied Biosystems 2720 Thermal Cycler (Foster City, CA, USA) was used to perform PCR. This procedure was performed with 4 ng of DNA and 1 µM of each primer with a fluorescent dye attached to the forward primer (Fw) (6-FAM: VVS3, VVMD7, VVMD28, VVMD36, VrZAG47, VrZAG62, VrZAG83, VvUCH11, and VvUCH19; HEX: VVS2, VVS29, VVMD6, VVMD27, VrZAG21, VrZAG79, and VChr19a; NED: VVMD5, VrZAG64, scu06vv, and VvUCH12) using the Applied Biosystems AmpliTaq DNA Polymerase kit (Foster City, CA, USA). The thermocycling programme was as follows: 95 °C, 5 min; 40 cycles (95 °C, 45 s; Ta 30 s (Table S3); 72 °C, 1 min 30 s), and 72 °C, 7 min.

2.5. Amplified Fragments Length Measurement

For fragment measurement plates preparation, amplification products were mixed with 20.5 µL of deionised formamide and 0.25 µL of GeneScan ROXTM 500 internal marker (Applied Biosystems, Foster City, CA, USA). Each plate content was denatured with a thermocycling regime at 95 °C for 3 min. Fragments were separated by capillary electrophoresis with an ABI PRISM 3730® genetic analyser (Applied Biosystems, Foster City, CA, USA). Peak Scanner Software (Applied Biosystems, Sparta, NJ, USA) was used to measure the amplified fragments.

2.6. Data Analysis

To assess the reliability of the 20 SSRs used, the GenA-lEx 6.5 software [32,33] was employed. This software allows the study of six parameters: number of different alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F), and the probability of identity (PI). GenAlEx 6.5 has also allowed us to rule out identities, i.e., genetically identical individuals, as well as to detect mutations. Assignment tests based on allele frequency [34], also available in GenAlEx 6.5, were used for the first time to confirm the accessions belonging to each subpopulation generated by Structure 2.3. [35,36]. For each accession, a logarithmic probability value was calculated for

each subpopulation using the allele frequencies of the respective subpopulations. An individual was assigned to the population with the highest logarithmic probability value. In addition, this software was also used to calculate populations' genetic differentiation using an analysis of molecular variance (AMOVA) with 999 dataset permutations for SSR genotypes, with the Fst (coefficient of genetic differentiation between populations) assuming the infinite allele model. Finally, two-dimensional principal coordinate analysis (PCoA) was used in GenAlEx 6.5 to further examine genetic relationships between populations based on the same SSR data, both for populations per se and for populations disaggregated by individuals. PCoA was based on the standardised covariance of genetic distances calculated for codominant markers.

Structure 2.3. software [35,36] has been used to assess population structure and identify crossbred individuals. This model-based software uses a Bayesian clustering method in which several ancestral populations (K) are assumed to be present, each characterised by a set of allele frequencies at each *locus*. Sample individuals are assigned to populations (clusters), or jointly to more populations if their genotypes indicate that they are admixed. All *loci* are assumed to be independent, and each population K is assumed to follow Hardy–Weinberg equilibrium. Posterior probabilities were estimated using the Markov chain Monte Carlo (MCMC) method. MCMC chains were run with a 100,000 burn-in period followed by 1,000,000 iterations using a model allowing for admixture and correlated allele frequencies. Structure was run at least ten times by setting K from 1 to 7 (1 to 9 for global varieties), and an average likelihood value, L (K), was calculated across all runs for each K. The mean log probability of the data for each K was calculated to determine the most appropriate number of clusters, and the value of K for which this probability was highest was selected. The ΔK was then calculated using the method proposed by Evanno et al. [37]. ΔK is a quantity based on the rate of change in the log probability of the data between successive K values.

Dendrograms and phylogenetic trees were constructed using the neighbour-joining method [38], using MEGA version 7 [39]. For the three-dimensional PCoA representations, the Matplotlib strategy was used using Python Data [40].

3. Results

The 87 grapevine shoots prospected were genotyped with the same 20 SSRs used in previous studies [6,16,41,42]. The aim was to compare the molecular profiles of SSRs (MP-SSRs) obtained with those that have been found and stored in a private research group database (TECNENOL: Grupo de Investigación en Tecnología Enológica).

3.1. SSR Polymorphism

Once the MP-SSRs of the whole population were obtained, identical profiles were searched for and, after the first data normalisation, a total of 41 individuals were eliminated (Tables S1 and S2). The remaining 46 unique MP-SSRs corresponded to 28 varieties of *Vitis vinifera* ssp vinifera. Thus, each variety would include individuals with variations with respect to the most widespread MP-SSR, but not the "sport" (colour or hairiness mutation so specific that molecular markers do not detect it), as they have the same MP-SSR.

To verify the goodness and efficiency of the 20 SSRs used, 6 parameters indicative of these traits were studied (Table S4). A total of 185 alleles (Na) were detected in this population, with a mean of 9.3 alleles. The SSR with the lowest number of alleles was VVS3 with 3 alleles, and the highest number was VVMD27 with 15 alleles. The mean number of effective alleles (Ne) was 5, ranging from 1.17 (VVS29) to 10.47 (VVMD27). The means of Ho and He were 0.796 and 0.737, respectively. The SSR VVS29 reached minimum values in both cases (Ho = 0.156 and He = 0.148). Likewise, the maximum value found for Ho was for VVS2 (0.978), while the highest value for He was shown by VVMD27 (0.904). Six SSRs had positive F values, although all of them were very close to 0 (VVMD6, VVMD27, VVMD28, VrZAG83, VvUCH11, and VvUCH19). Finally, the accumulative identity probability of

the SSR set was 9.4×10^{-23}, defining a range between 7.3×10^{-1} for SSR VVS29, and 1.7×10^{-2} for SSR VVMD27.

3.2. Grapevine Variety Analysis

Grapevine variety analysis for their characterisation and identification was carried out at two levels: at the MP-SSR level and at the lexicographical level. To this end, an exhaustive bibliographic review (books, scientific articles, and databases) was carried out to find information on Canary Islands varieties [6,16–18,41,42]. The Vitis International Variety Catalogue (VIVC) database [43] was also used to verify the unknown individuals.

Tables S1 and S2 contain all relevant information on each genotyped individual. Table S1 contains both the original information provided by the winegrower and the conclusive information found at after matching MP-SSRs and names in the TECNENOL [6,16,41,42] and VIVC [43] databases. Information is also provided on the variations detected in each allele, the presence of tri-alleles, or the similarity percentage to the closest genome in the TECNENOL database. Table S2 also shows the seven SSRs values that coincide with the international SSRs [43].

Focusing on the 87 individuals analysis (Table S1), it can be seen that the Bermejuela variety was the one with the most entries. There were a total of 11, of which 7 corresponded to Bermejuela (W), with 5 identities with respect to the most widespread MP-SSR, and 2 mutations (Bermajuelo del Echedo (VVS3-2 (mutation of this SSR in the second allele)) and Bermajuelo del puerto (VVMD36-1 (mutation of this SSR in the first allele)). The remaining four entries corresponded to the new "sport", Bermejuela rosada (Rs), with only Bermajuelo rosado del tesoro showing variation (VVS2-2). The cluster of the local Herreña (term referring to autochthonous from El Hierro island) variety, Verijadiego, was much more uniform. Of its eight components, four individuals showed a MP-SSR identical to the most widespread one, and the remaining four showed a MP-SSR with a mutation in one allele (VVS3-2). Similarly, the Portuguese variety Alfrocheiro, which grouped six accessions, showed little variability (four with a MP-SSR identical to the most widespread, and the other two with a mutation in VVS3-2). The Listan negro and Vijariego blanco varieties, each consisting of five accessions, showed high variability. The Listan negro variety was very uniform, with only two MP-SSRs (four components identical to the most widespread MP-SSR, and one individual with a variation in VvUCH11-2), while the Andalusian variety Vijariego blanco was so variable that no component identical to the most widespread MP-SSR was recorded. Thus, the accessions Vijariego blanco from El Hierro and Burra blanca vary in VVMD36-1; the accession Eusebia presented two variations (VVS3-2 and VVMD36-1); the accession known as Diego de El Hierro showed three variations (VVMD28-1, VVMD36-1, and a case of tri-allelism in the SSR, SCU06vv), and finally, the accession known as Diego de Frontera also varied in three alleles (VVS3-2, VVMD36-1, and VvUCH12-2). With four accessions each, there are the following variations: (a) Samarrinho and Trousseau noir, without variations; (b) Mollar cano with three individuals with MP-SSR identical to the most widespread and one with a variation in VVS3-2; (c) Albillo forastero with one accession with a MP-SSR identical to the most widespread and three individuals that presented the same mutation (VVS3-1); (d) the Listan prieto variety showed one accession with a MP-SSR identical to the most widespread, two entries mutated on one allele (VVMD28-1) and one entry mutated on two alleles (VVS29-2 and VVMD28-1); (e) the Portuguese variety Molar showed one accession with a MP-SSR identical to the most widespread, two entries mutated on VvUCH12-1, and one entry mutated on VVS29-2. With three accessions, the varieties Muscat of Alexandria, Verdello de El Hierro and Malvasia fina have been obtained. The first two had all their components identical to the most widespread MP-SSR, and the last one had a mutated component in VVMD28-2. There was a group of five varieties with two components each: (a) the varieties Malvasia Dubrovacka and Sumoll, with all their components identical to the most widespread MP-SSR; (b) the varieties Palomino fino and Isabella, with a variation in one accession from the former in VVS3-1, and the latter with a tri-allelic in this same SSR; finally, (c) the variety Airen, which

has all its accessions mutated in the same alleles (VVS3-2 and VVMD28-1). Finally, there is a group of eight varieties with only one representative and which do not present any variation with respect to the most widespread MP-SSR: the Spaniard south-west variety, Beba; the Lanzarote variety Uva de año, the Portuguese variety Verdelho branco, and the six new varieties described for the first time on the island of El Hierro (Uval piñero, Uvalero volcánico, Pinar negro, Seis de Carlos, Tesoro blanco, and Uval negro).

As noted in previous paragraphs, the 46 unique MP-SSRs (Table S2) found corresponded to 28 varieties, of which 6 were unknown. Of the remaining 22 varieties, 4 were Canary Islands varieties (Albillo forastero, Bermejuela, Listan negro and Uva de año) and, more concretely, 2 were varieties from the island of El Hierro already described by other authors (Verdello de El Hierro and Verijadiego) [17,18]; the remaining 16 were not from the Canary Islands archipelago. Regarding the latter, seven were Spanish (Airen (represented by a mutation), Beba, Listan prieto, Mollar cano, Palomino fino, Sumoll, and Vijariego blanco (represented by a mutation)), five were Portuguese (Alfrocheiro, Malvasia fina, Molar, Samarrinho, and Verdelho branco), and there were also the Greek Muscat of Alexandria, the French Trousseau noir, the Malvasia Dubrovacka of unknown origin (but located in the Balkan Peninsula), and the American variety corresponding to a direct producer hybrid (DPH), Isabella. A pink-coloured mutation ("sport") was also described for the first time for the Canary Islands variety Bermejuela, namely Bermejuela rosada. Rodríguez-Torres [18] also documented in his work another "sport" for the Bermejuela variety, namely Bermejuela tinta (black-violet), which was not found in this prospection. Eleven accessions were identified as "unknown", fifteen errors were detected, eighteen new mutations of known varieties were presented, and two mutations previously described on the island of Lanzarote were detected (Mollar bonilla corresponding to a mutation of the variety Mollar cano (VVS3-2) and Listan blanca chicharrera corresponding to a mutation of the variety Palomino fino (VVS3-1)) [42].

As far as variety names are concerned, 18 new names are proposed for the new mutations detected: Airen del pinar, Forastera de la isla Redonda, Baboso negro de Frontera, Bermajuelo del Echedo, Bermajuelo del puerto, Bermajuelo rosado del tesoro, Mierda de gallina, Listan negro del tesoro, Listan prieto chijo, Listan prieto herreño, Malvasia fina gabetera, Molar tintilla, Molar herreño, Verijadiego blanco de Frontrea, Vijariego blanco de El Hierro, Eusebia, and Diego de El Hierro y Diego de Frontera. In addition, one synonymous name registered for a given variety, used to name another variety, was detected. This is the case of the term Baboso blanco, used in El Hierro to refer to the Portuguese variety Samarrinho. Officially (VIVC), this term is a synonymy used to designate the white mutation of the French variety Trousseau noir, which is known under the name Bastardo blanco as the main name of this mutation ("sport" in this case). Five new synonymies are presented: Bermajuelo as a synonymy of El Hierro for the Canary Islands variety Bermejuela, Bermajuelo rosado de El Llano, Bermajuelo rosado, and Mulata rosada to name the new mutation described in this island for the first time (Bermejuela rosada), and Negra muelle as a new synonym of Listan negro. Finally, there is also a homonymous name in the case of the term Uval blanco, which is used in this island to name (as a synonym) both the Portuguese variety Malvasia fina and the local variety of this island, Verijadiego.

3.3. El Hierro Grapevine Population Genetic Structure

To carry out the genetic structure study of the accessions on El Hierro island, the Structure 2.3. programme was used carrying out an additional normalisation of the data. From the 46 unique MP-SSRs in Table S2, all individuals showing variations for the same variety were eliminated. Thus, only the most widespread MP-SSR was left, and in case there was none (Airen and Vijierego blanco), the individuals with the highest similarity to the most widespread MP-SSR were computed. In this sense, the el Hierro population consisted of 28 grapevine varieties. In order to know the best grapevine variety distribution in different populations (K), it was proposed that they be grouped into up to seven ancestral populations. Figure S1 shows the best distribution found after applying the correction of

Evanno et al. [37]. It can be seen that the best distribution corresponded to K = 3, i.e., it is proposed to group the 28 varieties into 3 ancestral populations. Figure 3a shows the 28 varieties from El Hierro distributed in 3 groupings. Applying the GenAlEx 6.5 assignment test for K = 3, it was seen that this distribution presented a goodness of assignment of 89%. In addition, it can be seen how each population groups their components into pure and admixed according to the q value. q is a measure of an individual's membership of a population based on its genetic similarity (percentage of its inferred genome belonging to the group [44]) and is ordered from highest to lowest (Table S5). This strategy allows a further normalisation of the data by allowing the detection and elimination of varieties with q-values < 85% (admixed), which would distort the final result of the study.

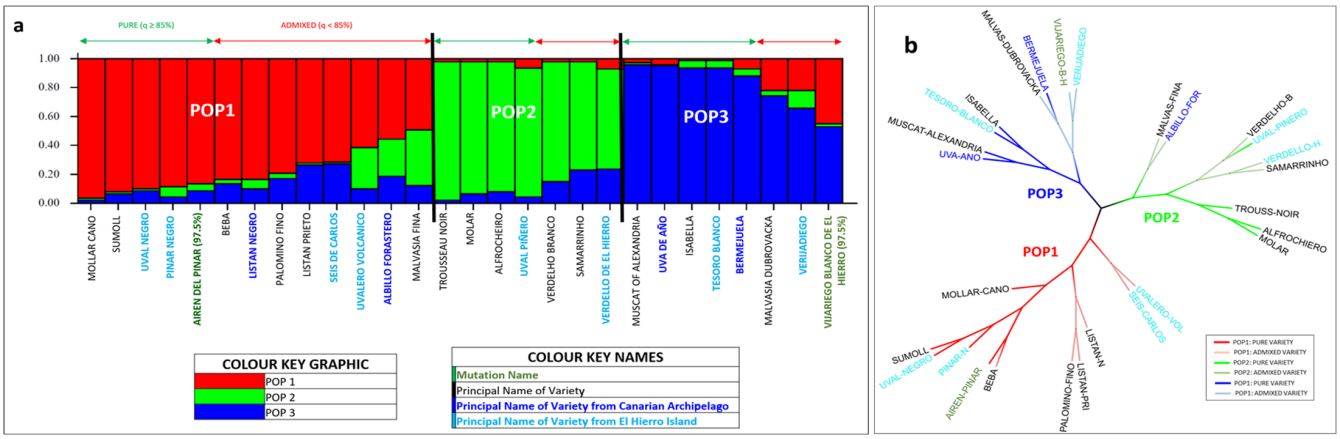

Figure 3. El Hierro grapevine varieties population (unique molecular profiles). (**a**) Structure 3.2 diagram: K = 3 distribution for pure and admixed individuals. (**b**) Phylogenetic tree of this distribution.

Thirteen varieties were grouped in POP1. Five were pure (q ≥ 85%; two of them from El Hierro) and the remaining were admixed. It can also be observed that 46% were Spanish varieties (seven varieties) and 8% were Portuguese (one variety). Additionally, 15% of the varieties were Canarian (two varieties) and 31% were exclusively from El Hierro island (four varieties). POP2 is a Portuguese group formed from seven varieties, four pure (57%) and three admixed (43%), of which two are Herreñas (one pure and one admixed). Finally, POP3 is shown with eight representatives with different origins, five pure (63%) and three admixed (37%). Figure 3b shows the phylogenetic tree for this population of 28 varieties. It clearly shows the three populations described and equidistant on three main branches. In each branch, the dichotomy between varieties considered pure and admixed can also be observed, with the exceptions of Uval pinero (pure variety of POP2) and Bermejuela (pure variety of POP3) which are grouped with the admixed varieties of their population.

The optimal representation of grapevine variety distribution from El Hierro by means of principal coordinates analysis (PCoA) required the elimination of the 14 admixed individuals from the population recommended by the Structure 2.3. program. Thus, the population of El Hierro was reduced to 14 pure representatives: (a) POP1 included the Spanish Mollar cano, Sumoll and a mutation of Airen, and Uval negro and Pinar negro from El Hierro; (b) POP2 included the French Trousseau noir, the Portuguese Molar and Alfrocheiro, and the Herreña Uval piñero; (c) POP3 featured the Greek variety Muscat of Alexandria, the Canary Islands varieties Uva de año and Bermejuela, the American DPH Isabella, the Malvasia Dubrovacka (Malvasia aromatica), and the variety Tesoro blanco from El Hierro. Figure 4 shows the two-dimensional (Figure 4a) and three-dimensional (Figure 4b) PCoA representations of this population.

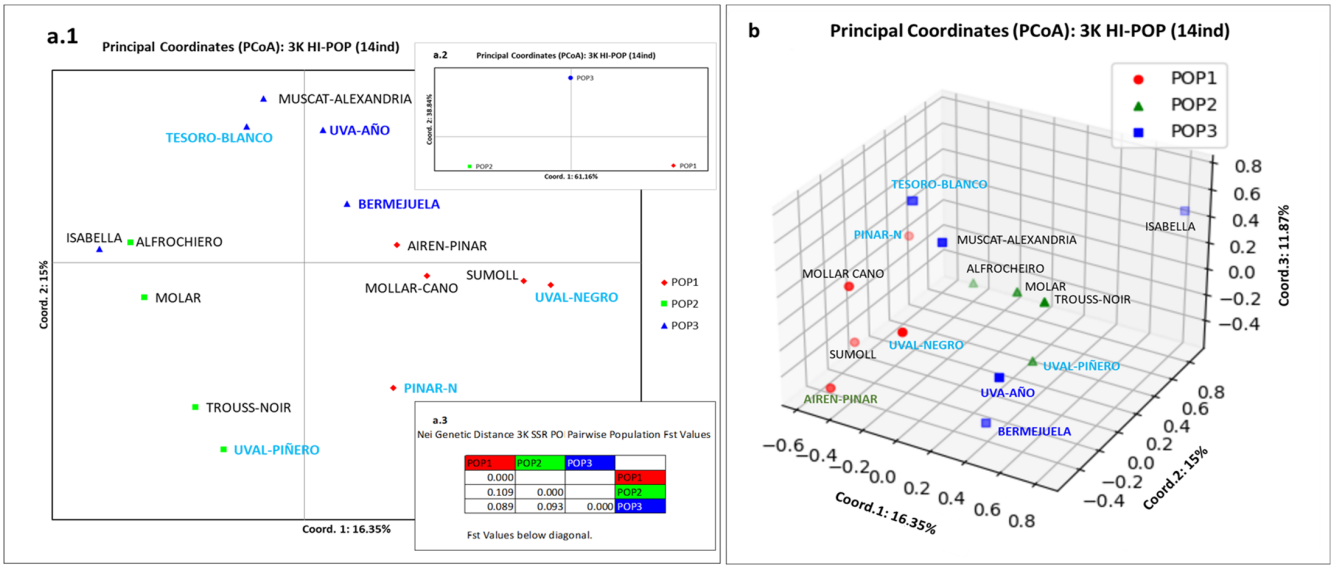

Figure 4. PCoA representations of the grapevine varieties population from El Hierro island normalised for K = 3. The names in navy blue correspond to Canarian varieties, the names in light blue correspond to varieties from el Hierro, and the names in black correspond to varieties from outside the archipelago. (**a.1**) Two-dimensional representation of the three populations by individuals, (**a.2**) two-dimensional representation of the three populations from el Hierro by population, and (**a.3**) values of the Fst statistic for each population. (**b**) Three-dimensional representation of the three populations by individuals.

Figure 4a.1 shows the population of pure varieties from El Hierro. It can be seen how coordinate 1 (with a goodness of fit of 16.35%) separates the Spanish varieties from the non-Spanish varieties, while coordinate 2 (with a goodness of fit of 15%) divides the varieties with an Eastern Mediterranean influence from those with a more Central European or Hispanic influence. In this way, POP1 is practically located in the lower right quadrant. Similarly, POP2 is located in the lower left quadrant, and POP3 occupies the central area of the upper quadrants. Other aspects to highlight are the position of the variety Tesoro blanco, which is very separate from the rest of the varieties on the island of El Hierro. The position of the DPH Isabella is also notable and very distant from the rest of the POP3 members. This arrangement of populations is also reproduced in Figure 4a.2, (representation of the populations with all their individuals grouped together). In addition, Figure 4a.3 shows the result of the analysis of molecular variance (AMOVA). Thus, the most distant populations are POP1 with respect to POP2, followed by POP2 with respect to POP3, and the closest are POP1 with respect to POP3. Finally, it should be noted that with one more dimension, DPH Isabella is distant from the rest, and the large dispersion of POP3 and the marked position of the varieties from El Hierro, which are widely scattered among them, are evident (Figure 4b).

3.4. El Hierro Grapevine Population Genetic Structure Respect to the World Population

The aim of this section is to see the extent of the uniqueness of the population of varieties on the island of El Hierro as a whole. To this end, in addition to the six new varieties described in this work (Uval piñero, Uvalero volcánico, Pinar negro, Seis de Carlos, Tesoro blanco, Uval negro), another five varieties described in previous works by different researchers will be added to the population of El Hierro. These are the varieties Burra volcanica [16], Huevo de gallo [16,18], Verdello de El Hierro [17,18], Verijadiego [16–18] and Verijadiego negro [16]. Therefore, from now on, the Herreña population will be formed by 11 varieties, which will be compared with a world population from the TECNENOL database, formed by 297 varieties (unique MP-SSRs of *Vitis vinifera* ssp vinifera) from 22 countries and analysed with the same 20 SSRs [6,16,41,42].

The procedure followed has been the same as in the previous section but involves trying to distribute a population of 308 unique MP-SSRs in up to 9 ancestral populations using Structure 2.3. Figure S2 shows the best distribution for the population under study which, in this case, corresponds to the value of K = 2. Figures S3 and S4 show the 308 varieties distributed in the 2 ancestral populations as a function of q. In this case, 181 MP-SSR (59%) were grouped in POP1, with a predominance of Italian (54 individuals out of a total population of 72 Italian (54/72)), French (44/49), and, to a lesser extent, Spanish (28/105), Portuguese (16/22), and Greek (11/14) varieties. This grouping had 161 pure components (89%), of which 51 were Italian, 39 were French, 24 were Spanish, 12 were Portuguese, and 11 were Greek, as well as 20 mestizos (11%), of which 3 were Italian, 5 were French, 4 were Spanish, and 4 were Portuguese. The remaining nationalities were represented mainly by Balkan, Eastern Mediterranean, and Central European countries. The remaining 127 components (41%) were placed in POP2, with a predominance of Spanish (77/105), Italian (18/72), Portuguese (6/22), and Greek (3/14) individuals. Of all the components of this group, 97 varieties were pure (76%), with 66 Spanish, 10 Italian, 2 Portuguese, and 3 Greek individuals, as well as 30 admixed varieties (24%) with 9 Spanish, 8 Italian, and 4 Portuguese varieties. In POP2, all the Canary Islands varieties were grouped together and, therefore, so too were the grapevine varieties from El Hierro, so that 11 varieties from Lanzarote, 11 varieties from El Hierro, and 7 from the rest of the Canary Islands were pure; the remaining two, the Albillo criollo variety from the island of La Palma and the Malvasia alistanada fina from Lanzarote, were found to be located next to the admixed individuals. It should be noted that, while most of the El Hierro varieties are very close together in the circular dendrogram (Figure S4b), there are two, the Uval piñero variety and the Tesoro blanco variety, which are far away from the main group. The Uval piñero variety is found in the first of the three main branches originating this circular dendrogram, while the Tesoro blanco variety is located in the first of the two branches originating POP1.

The identification of the 50 admixed individuals allowed us to perform the final data standardisation, and these were eliminated from the study. Thus, the population was left with 258 varieties. Applying the consequent assignment test, the goodness of fit of each variety in the two proposed populations (K = 2) was 100%. Figure 5a shows the Structure 2.3. diagram with pure and admixed individuals, and Figure 5b shows the circular dendrogram of the pure individuals. If the circular dendrograms are compared, with (Figure S4b) or without (Figure 5b), the overall result is practically the same. However, at a grapevine variety location level, small changes can be perceived due to the relocation of the pure individuals once the admixed individuals have been eliminated.

The two-dimensional and three-dimensional PCoA representation is presented in Figure 6. It should be noted that in order to analyse the uniqueness of the El Hierro and Canary Islands grapevine populations, these were extracted from POP2, preserving all their pure components (17 varieties for the Canary Islands (IC) and 11 varieties for the island of El Hierro (HI)).

In Figure 6(a.1,a.2) the clear separation between POP1 and POP2 with IC and HI is shown. In fact, it is coordinate 1 (with a goodness of fit of 5.69% for individuals, and 64.82% for populations), which practically separates them. The effect of coordinate 2 is not visible in Figure 6a.1 (with a goodness of fit of 3.70%), but it is visible in the population plot with a goodness of fit of 19.91% (Figure 6a.2), leaving the El Hierro population alone in the lower left quadrant. Figure 6a.3 confirms that the HI population is the most distant from the rest, followed by the IC population. The slight overlap of POP2, IC (located in the innermost zone) and HI (located in the outermost zone) shown in Figure 6a.1 is not reflected in Figure 6a.2, where these three populations occupying the left quadrants are perfectly separated (see also Figure 6a.3). Another fact to take into account is the distant position, not only with respect to HI but also to IC and POP2, of the variety Tesoro blanco (unknown number 6 [18]), which is practically located in POP1. Figure 6b shows the three-dimensional representation of PCoA for the population under study. This image also shows a separation between POP1 (practically located in the lower and innermost part) and POP2 (practically

located in the upper front part). On the other hand, for the populations IC and HI, which are in the front part of the graphical representation, IC is in the lower part and HI in the upper and more external part. The HI variety Tesoro blanco is hidden by individuals of POP1 and POP2 (lower centre-right). However, Figure 7 (corresponding to another angle of the same three-dimensional representation above) shows the distant positions of both the Tesoro blanco and the Uval piñero varieties.

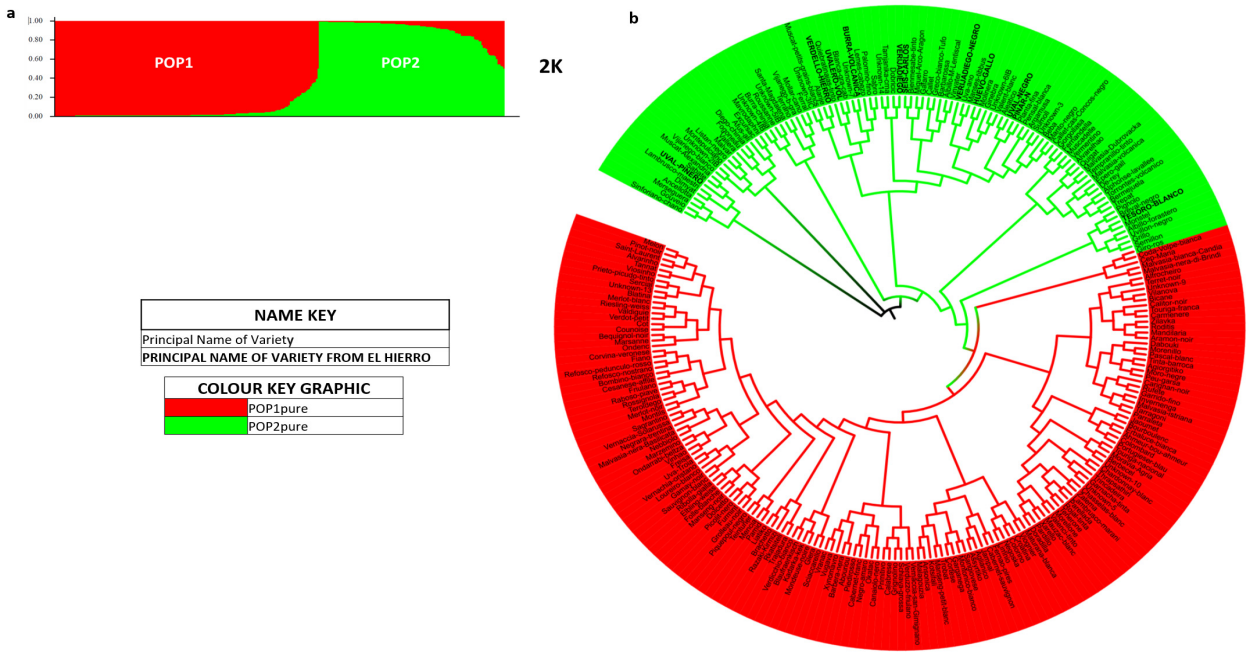

Figure 5. World population (258 individuals) distributed in two populations. (**a**) Graphical representation of K = 2 according to Structure 2.3. (with pure and admixed individuals). (**b**) Circular neighbour-joining dendrogram of the world population 258 pure individuals, highlighting the location from El Hierro (in capital letters and bold).

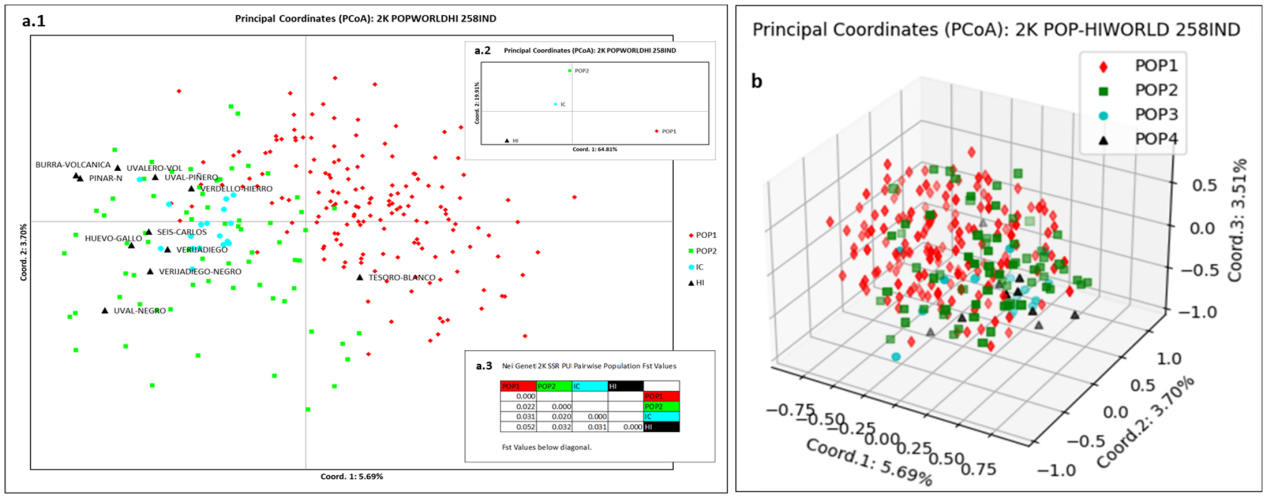

Figure 6. PCoA representation of the grapevine variety population from El Hierro, Canary Islands, and the world normalised to K = 2. (**a.1**) Two-dimensional representation of the four populations by individuals, (**a.2**) two-dimensional representation of the four populations by population, and (**a.3**) values of the Fst statistic for each population. (**b**) Three-dimensional representation of the four populations by individuals (in this representation, POP3 corresponds to IC, and POP4 corresponds to HI).

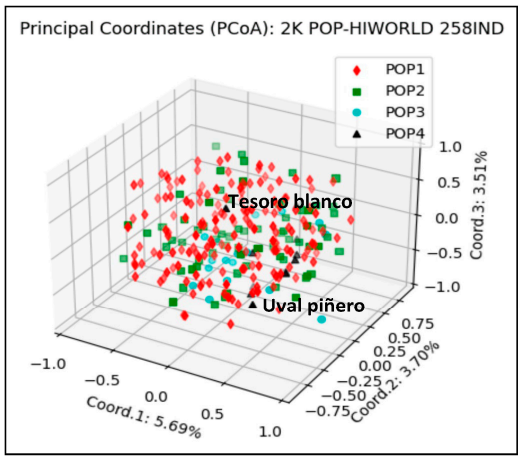

Figure 7. Three-dimensional representation of the four populations by individuals (in this representation, POP3 corresponds to IC and POP4 corresponds to HI). Visualisation under a different angle from the one presented in Figure 6b. The two El Hierro grapevine varieties with positions further away from the rest are highlighted.

In order to confirm the uniqueness observed for the population of varieties on El Hierro island, a study was carried out in which a geographical strategy was introduced as the main criterion. The aim was to group the varieties to create populations according to the country of origin registered in VIVC [43]. It was found that there were countries with a very low number of components, so the strategy of creating populations was extended to geographical areas [6,16,41,42,44,45]. Specifically, seven populations were created: the EASTMED-CAU population (Algeria, Cyprus, Georgia, Israel, Lebanon, Tunisia, and Turkey), the BP population (Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Serbia, Slovenia, and Montenegro), the ITA population (Italy), the FRA-CEU population (Austria, France, Germany, Hungary, and Switzerland), the IP population (Spain and Portugal), and the IC and HI populations. Figure S6a shows the neighbour-joining circular dendrogram of the 308 individuals of the world population grouped in populations corresponding to the 7 defined geographical areas. Once the 7 populations containing the 308 varieties were grouped, an assignment test was performed. A 60% goodness of fit was found. In Figure S6a,b, it can be seen how three main branches are formed. The first one is where the IC and HI varieties are placed, together with a few IP varieties. In the second one, almost all the IP varieties are placed, and the third branch holds the rest of the world populations. It can also be observed how the first branch is subdivided and, in the third subdivision, gives rise to a differentiation into two sub-branches, one containing exclusively IC varieties, and the other containing all the HI varieties, some IC admixed, and some peninsular varieties (pure (all unknown assigned to IP) and admixed (Mollar cano and Molinera)). In Figure S6a, it can also be seen how in El Hierro population, there are two admixed varieties (Tesoro blanco and Seis de Carlos) and the admixed varieties in the IC population are Vijariego blanco de la granja, Diego chinija, Sabro, Burra chinija, and Breval negro. Another aspect to take into account is the location of the three admixed varieties of IC in the third large branch together with other PI admixed types. These are Albillo forastero, Albillo del monte Lentiscal, and Bienmesabe tinto. Finally, another fact to note is regarding the variety from La Palma Island, Albillo criollo (pure), that is displaced to the FRA-CE population, and is located with a group of admixed grapes from this same cluster. The composition of each of these seven groups can also be seen in detail in Figure S5.

The low goodness of fit of the assignment test forced the misplaced varieties (which were mostly the result of admixture) to be discarded so that the world population was reduced to 184 varieties. A further assignment test was carried out to check the goodness of fit of the new distribution. With these new conditions, the goodness of fit reached 92%. Figure 8a,b shows a much sharper and more precise distribution than the previous one,

where the first of the three large branches corresponded to the EASTMED-CAU and BP populations, the second branch contained the ITA and FRA-CE populations, and the last branch contained the IP, IC, and HI populations.

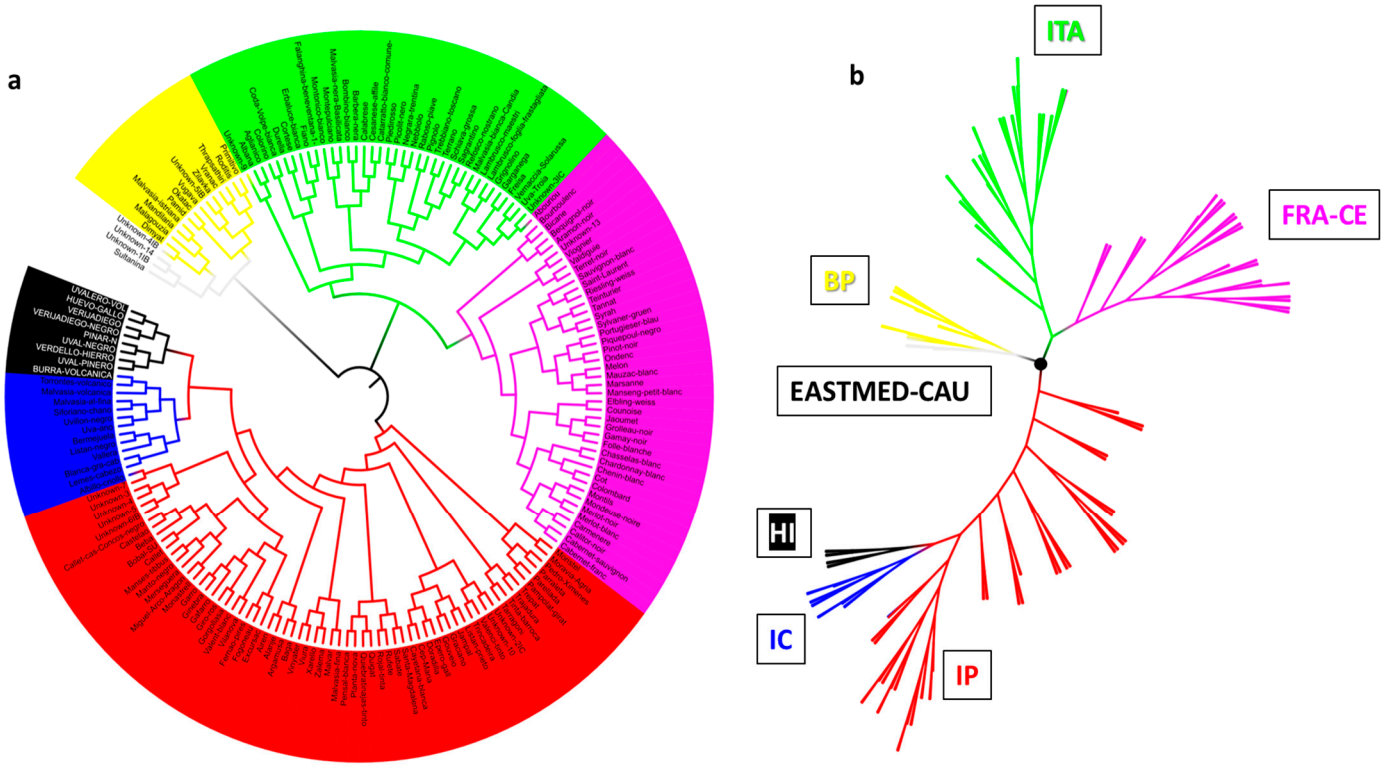

Figure 8. World population (184 individuals) distributed in populations corresponding to seven geographical areas. (**a**) Circular neighbour-joining dendrogram of the 184 pure individuals of the world population, highlighting the location of the varieties from El Hierro. (**b**) Phylogenetic tree of the distribution of these seven populations with all their individuals.

The PCoA representation of these populations is shown in Figure 9. Figure 9a.1 shows how IC and HI both overlap with IP, but there is differentiation between the first two. Moreover, it is coordinate 1 (with a goodness of fit of 6.19%) that separates these populations of Spanish origin from the ITA and FRA-CE populations. In this image, the EASTMED-CAU and BP populations are blurred in the centre, occupying all the quadrants. The discrimination made by coordinate 2 (with a goodness of fit of 4.07%) in this figure is not clear. The population representation itself (Figure 9a.2), is much more decisive. In this way, coordinate 1 again differentiates (with a goodness of fit of 46.36%) the Spanish populations from the rest, and coordinate 2 (with a goodness of fit of 17.6%) separates the EASTMED-CAU, IP, and HI populations from the rest. Thus, only IC is located in the upper right quadrant, IP and HI are in the lower right quadrant are IP and HI, and in the upper left quadrant, very close to coordinate 2, are ITA and FRA-CE, and BP is a little further away. Finally, EASTMED-CAU is in the lower left quadrant and very far from the axis. These positions are confirmed by the values of the Fst statistic shown in Figure 9a.3. In Figure 9b, there is a slight differentiation between the IC and HI populations with respect to IP, as the third dimension raises most of the IC and HI individuals above the position of most of the IP varieties. The behaviour of the rest of the populations is similar to that shown in the two-dimensional plot.

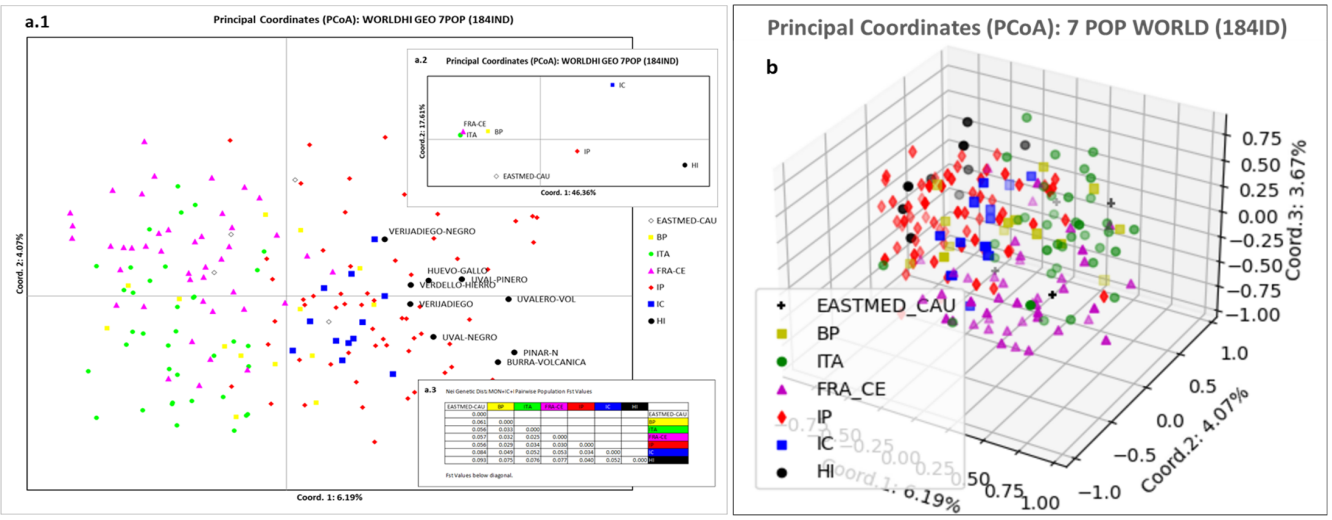

Figure 9. World grapevine varieties population PCoA representation (184 individuals) according to the geographical criterion. (**a.1**) Two-dimensional representation of the seven populations per individuals; (**a.2**) two-dimensional representation of the seven populations per population; (**a.3**) values of the Fst statistic for each population. (**b**) Three-dimensional representation of the seven populations per individuals.

4. Discussion

4.1. SSR Polymorphism

The analysis of grapevine varieties from El Hierro island produced interesting results; however, it is first necessary to assess the goodness of the 20 SSRs that have been used. These microsatellites have been used in all of TECNENOL's work [6,16,41,42] and, in this way, this research group has been creating its own database, which allows for the exhaustive and precise comparison of new MP-SSRs. So far, this SSR kit has proven to be efficient and effective. Table S4 shows a summary of the results of the main statistical parameters obtained for this study. It should be taken into account that the comparison with other studies may be very approximate, as the number of SSRs used, the number of samples analysed, and the closeness of the population samples to be analysed may substantially vary the final results of the studies. [46,47]. The total Na obtained from El Hierro population was 185 alleles. These results are slightly lower than those obtained in the study on the island of Lanzarote [42], because more than twice as many samples (223 vs. 87) were analysed in Lanzarote for the same SSRs. In the case of the Balearic and Canary Archipelagos prospection, approximately the same number of samples with the same SSRs were analysed [6,16], but significantly higher values were also found with respect to HI. Obviously, this was because it was a group of islands with different and more numerous local varieties than a single island. The several studies carried out on the mainland [48–50], always give higher results due to the greater diversity of their samples. On the other hand, the mean Na value was not significantly different from studies on varieties from Turkey [48] and Croatia [49], but lower than studies on the Balearic and Canary Islands [6,16]. The mean genetic diversity index, or He, was 0.737, within the range of most of the studies consulted and slightly higher than the one found in Lanzarote, as its population was more uniform [42]. Fourteen SSRs were found with an F of less than 0.01, meaning that they have a small excess of heterozygosity, reaffirming the consistency of the homozygous individuals. The accumulative PI was also within the expected range (9.4×10^{-23}) for such a study, indicating that this SSR kit was able to guarantee that two individuals with the same MP-SSR at all *loci* were the same individual (except for the "sport" loci).

The best SSR for this population were VVS2 (Na: 13; Ne: 6.23; He: 0.840; F: −0.165; PI: 4.5×10^{-2}), VVMD5 (Na: 12; Ne: 8.42; He: 0.881; F: −0.034; PI: $2{,}6 \times 10^{-2}$) and y el

VVZAG79 (Na: 12; Ne: 6.17; He: 0.838; F: −0.087; PI: 4.3×10^{-2}). The least informative were VVS3 (Na: 3; Ne: 2.00; He: 0.500; F: −0.154; PI: 3.6×10^{-1}), VVS29 (Na: 5; Ne: 1.17; He: 0.148; F: −0.054; PI: 7.3×10^{-1}), and VVMD6 (Na: 5; Ne:3.59; He: 0.721; F: 0.076; PI: 1.2×10^{-1}). At this point, it can be concluded that this SSR kit continues to be suitable for characterising and identifying samples from El Hierro island.

4.2. Grapevine Varieties Analysis

Regarding grapevine population analysis, sample uniformity has to be emphasised. In the first data standardisation, 47% of the individuals were discarded because they were identical to other samples of the population. The 46 individuals from El Hierro with unique MP-SSRs were in turn reduced to 28 varieties (28 individuals). This fact indicated that 43% of the population of unique MP-SSRs (20 individuals, as there are 2 mutated individuals that are taken as representatives of the variety, as this collection does not have the generic MP-SSR) were mutations and, therefore, variability within a vine variety (intra-varietal variability) was detected. When working with 20 SSRs, 40 alleles are being compared. If a variation in a MP-SSR is detected in one allele, it is considered a mutated individual with a similarity of 97.5% with respect to the most widespread MP-SSR; when the variations reach two alleles, it is considered a mutated individual with a similarity of 95%; when the variation is three alleles, the individual has a similarity of 92.5%, and the similarity will be 90% when the MP-SSR varies in four alleles or 87.5% when the mutation reaches five alleles. From six variations onwards, it is already considered a new variety. This arbitrary delimitation is based on the works of Ibañez et al. [51] (SSR (2 alelos/26) 92%)), Vélez [52] (SSR (2 alelos/18) 89%)) y Cabezas et al. [53] (SNP, 90%). In Tables S1 and S2, all this intra-varietal variability is detailed, which not only reaches the typical numerical variation with respect to one of the two alleles of an SSR, but in this population, two cases of tri-allelism have also been described. These are the accession entered under the name Mierda de gallina (whose prime name (PN) in the VIVC is Isabella) and the sample entry under the name Diego de El Hierro (PN: Vijariego blanco). Since the beginning of the century, several authors have described the appearance of a third (or even a fourth) allele in a given SSR, indicating cases of hybridisation or chimerism [54,55]. For the 20 cases of individuals showing variations in their MP-SSR, 2 match a molecular profile already described in the Lanzarote prospection [42]. For this reason, they already have a name proposed to be included in VIVC in the corresponding publication; however, for the remaining 18 individuals, 18 particular names are proposed to name them and to be included in the world database. This proposal is made because VIVC provides the names of Pinot meunier for a mutation of Pinot noir, Chasselas cioutat for a mutation of Chasselas blanc, and Bastardo blanco for a colour mutation (sport) of Trousseau noir. In addition, 11 accessions have been identified and entered with the name "unknown". Fifteen errors have been detected (very possibly due to the vine grower's lack of knowledge), six new varieties have been characterised and identified for the first time (Uval piñero, Uvalero volcánico, Pinar negro, Seis de Carlos, Tesoro blanco, and Uval negro), and a new colour mutation for the Bermejuela variety (Bermejuela rosada) that already possessed a sport, Bermejuela tinta, has been identified [18]. It is also proposed to incorporate five new synonymous names (Bermajuelo, Bermajuelo rosada, Mulata rosada, Bermajuelo mulato de El Llano y Negramuelle), plus the term Baboso blanco as a new synonym of the Portuguese grapevine variety Samarrinho. Finally, the detection of a homonym for the Uval blanco grapevine variety, which would be one of the new synonyms used on El Hierro to name the varieties Verijadiego and Malvasia fina, is proposed to incorporate for both varieties.

4.3. El Hierro Grapevine Population Genetic Structure

Representatives of 28 varieties have been found on El Hierro island, of which 8 correspond to local grapevine varieties (Tabla S2). Of these eight varieties, six have MP-SSR described for the first time, while the remaining two have been described before [17,18]. El Hierro grapevine varieties have been distributed among three clusters (Figures 4 and 5

and Table S5), with four pure and four admixed placed as follows: four in POP1 (two pure vs. two admixed), two in POP2 (one to one), and two in POP3 (one to one). In POP1, mainly Spanish and Canary Islands grapevine varieties were grouped together, with the exception of the Portuguese admixed, Malvasia fina, a cross between the Heben variety (very widespread in the northern half of the Iberian Peninsula) and the Portuguese Alfrocheiro (located in POP2). POP2 (green colour) is a Portuguese group, with the exception of the French Trousseau noir (very widespread in Portugal and known as Bastardo negro). All the known components of POP2 have their pedigrees described, and in all of them the variety of Central European origin (unknown) Savaning blanc appears as one of its progenitors. It would also form part of the second generation of progenitors of the admixed varieties and one from POP1, namely Albillo forastero (Palomino fino × Verdelho branco) and Malvasia fina (Heben × Alfrocheiro), respectively, with a large green area in its genome, as can be seen in Figure 3. Finally, POP3 groups together other varieties, such as Muscat of Alexandria or Malvasia Dubrovacka, which means that the group will have a strong influence from the Eastern Mediterranean area. In addition, an American DPH is observed (Isabella variety) a cross between a *Vitis labrusca* and the Meslier petit vinifera (Heunisch weiss × Savaning blanc). The Savaning blanc variety, so common in Portuguese varieties, seems to have entered the Iberian Peninsula via the "Camino de Santiago". [56], leaving a remarkable progeny in addition to the Central European trace in the peninsular genetic profiles. In this study, the presence of this variety represents the genesis of a grouping (POP2).

Figure 4 shows how the Isabella variety moves away from the POP3 group and from the *vinifera* in general, due to the influence of *Vitis labrusca*, but remains close to POP2, due to the presence of the Savaning blanc variety in the second generation of parents. In short, this sample of varieties from El Hierro represents the history of the introduction of the domesticated vine on this island from the Spanish colonisation to the successive incorporations of Portuguese settlers from the archipelagos of the Azores and Madeira [17,57]. Thus, the most genuinely Spanish-influenced varieties are Uval negro and Pinar negro, the admixed Seis de Carlos and Uvalero volcanico, as they show a marked influence from the Eastern Mediterranean and Central Europe (via Portugal), respectively. El Hierro grapevine varieties present in POP2 and, therefore, with a strong Portuguese (Central European) influence are the Uval piñero (pure) and the admixed Verdello de El Hierro with a strong Eastern Mediterranean influence. The pure POP3, Tesoro blanco, and the admixed Verijadiego (with a strong peninsular and marked Central European influence) show in their MP-SSR traces of the influence of the Eastern Mediterranean on the peninsular varieties, which later, as can be seen, was transferred to the Canary Islands and the island of El Hierro.

4.4. El Hierro Grapevine Population Genetic Structure with Respect to the World Population

The objective of this section is to show the uniqueness of El Hierro population, compared to the world population. As has already been mentioned in other articles the introduction of vines in the Canary Islands dates back to the 15th century, so the uninterrupted evolution of the vine in this new island ecosystem is older than 500 years old [57]. Thus, the goal is to check whether adaptation to the island's soil and climatic conditions and both natural and anthropogenic selection have left an identifying mark on these varieties in their MP-SSRs. The first strategy adopted was to group the varieties by genetic proximity. The 11 varieties from El Hierro (new: Uval piñero, Uvalero volcánico, Pinar negro, Seis de Carlos, Tesoro blanco, Uval negro; already published: Burra volcánica, Huevo de gallo, Verdello de El Hierro, Verijadiego and Verijadiego negro) were compared with the remaining 297 individuals from the TECNENOL database from 22 countries. After Structure 2.3. allowed us to detect and eliminate the admixed varieties ($q < 85\%$) of the 2 ancestral groups formed, the 258 pure individuals of the world population were represented by a neighbour-joining circular dendrogram (Figure 6b). It can be seen how all the population from el Hierro was located in the fourth arm of POP2, and it can also be seen how the

varieties Tesoro blanco and Uval piñero were located at a distance from the rest of the group. In this figure, the IC, HI, and POP2 populations were in the same grouping and highlighted in green, which was logical since the IC and HI populations had been extracted from POP2 (a grouping made up mainly of Spanish varieties). In the individual representation by PCoA (Figure 6a.1 and Figure 7b) a slight differentiation between CI and HI is shown, while when the representation is visualised by populations (Figure 6a.2) a clear differentiation between POP2, CI, and HI is seen, with the latter occupying a quadrant by itself. This result is validated in Figure 6a.3, where results are presented for the Fst statistic, which takes into account the identity of alleles in the infinite allele model (IAM) when populations are compared pairwise (AMOVA). This is a method used to estimate the differentiation between populations from molecular data. Therefore, it can be concluded that the HI population has its own uniqueness and that there is, above all, a special distinction between the Tesoro blanco variety and the rest of the grapevine varieties from El Hierro.

Another aspect to consider is the low percentage of the goodness of fit of the graphical representations by individuals using PCoA [4,58]. This is because by using 20 SSRs and having 2 alleles each, we are using 40 numerical data to represent an individual on a graph. It would then have to be possible to represent a graph with 40 dimensions, and this is not possible. The reduction to two dimensions, or at best to three, is what reduces the reliability of the coordinates of the graph. It then becomes necessary to talk about trends.

In order to support the thesis on the uniqueness of El Hierro grapevine varieties, another strategy was developed. The next step was to look at the behaviour of the population studied under a geographical component. The 308 varieties were, thus, grouped into 7 groups corresponding to 7 different geographical areas, according to the country of origin in the VIVC database. The corresponding assignment test was carried out, and a goodness of fit of 60% was achieved. Figures S5 and S6 show the distribution of the resulting populations and which varieties were well assigned and which were not (admixed). Once the varieties misassigned by admixture were removed, the world population was reduced to 184 individuals with a goodness of assignment of 92%. It should be noted that under these conditions the variety Tesoro blanco was not well assigned and was consequently removed, which reinforces the idea that it is a markedly admixed variety, while the variety Uval piñero was retained within the group of varieties from El Hierro. Figure 8, both in the circular diagram and in the phylogenetic tree, confirms the uniqueness of both the Island of El Hierro and the Canary Archipelago, occupying a branch by themselves that divides, defining the two populations. This behaviour is also observed in PCoA representations, whether performed by individuals (Figure 9a.1) or by populations (Figure 9a.2), either in two (Figure 9a.1) or three dimensions (Figure 9b). In all cases, the trend of the El Hierro grapevine population is to differentiate itself not only from the Canarian archipelago population, but also from the other populations. Once again, Fst (Figure 9a.3) confirmed the uniqueness of the population of varieties from El Hierro, showing the highest values of the matrix compared to the rest of the populations. The notable exception will be that it is closer to the IP population than to the IC population, which indicates its greater influence from the Iberian Peninsula compared to the Canary Islands.

5. Conclusions

After carrying out the prospection, obtention, and analysis of data, the main conclusions obtained are as follows. On the one hand, the SSR kit that TECNENOL has been using proved to be suitable to continue with studies of this nature, and also allowed the presentation of six new varieties: Uval piñero, Uvalero volcánico, Pinar negro, Seis de Carlos, Tesoro blanco, Uval negro, and a new rose "sport" of Bermejuela (W). Two other interesting aspects are the fact that all the entries of individuals with the name Pedro Ximenez corresponded to the variety Albillo forastero and that all the accessions registered with the name Baboso blanco turned out to be the minority Portuguese variety Samarrinho. Fifteen errors were detected in total. Eleven varieties were identified that were unknown

to the vine growers, and twenty individuals with variations (mutations) were found, of which two had already been described in a previous prospection in Lanzarote Island.

It is proposed to incorporate the 7 new names of the new identified varieties and the "sport" into the VIVC database, in addition to the 18 names of the detected mutations (Airen del pinar, Forastera de la isla Redonda, Baboso negro de Frontera, Bermajuelo del Echedo, Bermajuelo del puerto, Bermajuelo rosado del tesoro, Mierda de gallina, Listan negro del tesoro, Listan prieto chijo, Listan prieto herreño, Malvasia fina gabetera, Molar tintilla, Molar herreño, Verijadiego blanco de Frontrea, Vijariego blanco de El Hierro, Eusebia, and Diego de El Hierro y Diego de Frontera), 5 new synonyms (Bermajuelo, Bermajuelo rosado de El Llano, Bermajuelo rosado, and Mulata rosada y Negra muelle), the new synonym for Samarrinho (Baboso blanco), and, additionally, the term Uval blanco for the grapevine variety Malvasia fina y Verijadiego, which has turned out to be the only case of homonymy.

The El Hierro grapevine population has a marked influence from the islands in its MP-SSR, and basically has three major sources of influence: the Iberian Peninsula through the Spanish varieties, Central Europe through the Portuguese varieties (Savaning blanc) and the east part of the Mediterranean Sea (Muscat of Alexandria and Malvasia Dubrovacka). Finally, it is necessary to highlight the case of the Tesoro blanco variety. This variety is significantly different from the rest of the El Hierro population, presenting a marked influence from the Eastern Mediterranean. For all of the above, El Hierro appears to have a unique population, not only worldwide, but even among the rest of the varieties of the Canary Islands archipelago. These results once again support the proposal that the Canary Islands and the El Hierro island are one of the few centres of biodiversity on our planet.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9121297/s1, Table S1: Original and conclusive information on 87 accessions from El Hierro. Similarity to the nearest genome (TECNENOL database); Table S2: List of the 46 unique profiles belonging to the population of the island of El Hierro. Values of the 7 international SSRs; Table S3: SSR groups by annealing Temperature (Ta); Table S4: Characterization of the twenty microsatellite markers used in this study; Figure S1: The 4 steps of the graphical method of Evanno et al. (2005) allow the estimation of the true number of ancestral K groups for a population of 28 varieties from the El Hierro collection; Table S5: Genetic structure of the El Hierro population. Distribution K = 3 (individuals belonging to each group or population); Figure S2: The 4 steps of the graphical method of Evanno et al. (2005), allow the estimation of the true number of ancestral K groups for a population of 308 varieties from the TECNENOL database; Figure S3: Genetic structure of the world population. Distribution K = 2 (Individuals belonging to each group or population). Detail of the proportion of pure and admixed individuals as a function of of q value. Nationalities that make up each group. El Hierro grapevine varieties are highlighted in red; Figure S4: World population (308 individuals) distributed in 2 populations. (a) Graphical representation of K = 2 according to the Structure 2.3. program. (b) Neighbour-joining circular dendrogram of the 308 individuals of the world population, highlighting the pure and admixed individuals, as well as the location of the El Hierro grapevine varieties (indicator arrows); Figure S5: Genetic structure of the world population. Distribution in 7 geographical areas. Detail of the proportion of well-assigned (pure) and misassigned (admixed) individuals. Nationalities comprising each of the groups: EASTMED-CAU (Algeria, Cyprus, Georgia, Israel, Lebanon, Tunisia, and Turkey), BP (Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Serbia, Slovenia, and Montenegro), ITA (Italy), FRA-CEU (Austria, France, Germany, Hungary, and Switzerland), IP (Spain and Portugal), IC (Canary Archipelago), and HI (El Hierro Island); Figure S6: World population (308 individuals) distributed in populations corresponding to 7 geographic areas. (a) Circular Neighbour-joining dendrogram of the 308 individuals of the world population, differentiating the well-placed individuals (pure) from the admixed (poorly placed), and highlighting the location of El Hierro grapevine varieties (indicator arrow); (b) phylogenetic tree of the distribution of these 7 populations with all their individuals.

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