OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By IVES International Viticulture & Enology Society

DOI: https://doi.org/10.20870/oeno-one.2022.56.3.5519

OPEN ACCESS

ORIGINAL RESEARCH ARTICLE

Molecular characterisation of the current cultivars of *Vitis vinifera* L. in Lanzarote (Canary Islands, Spain) reveals nine individuals which correspond to eight new varieties and two new sports

Francesca Fort*[1], Gemma Marsal[1], Josep Maria Mateo-Sanz[2], Violeta Pena[1], Juan Miguel Canals[1] and Fernando Zamora[1]

[1] Grup de Tecnologia Enològica (TECNENOL). Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Universitat Rovira i Virgili. Campus Sescelades. C/ Marcel·lí Domingo, 1. E-43007-Tarragona, Spain
[2] Grup CRISES. Departament d'Enginyeria Informàtica i Matemàtiques. Universitat Rovira i Virgili. Av. Països Catalans, 26. E-43007-Tarragona, Spain

*correspondence:
mariafrancesca.fort@urv.cat

Associate editor:
Georgios Merkouropoulos

Received:
21 April 2022

Accepted:
3 July 2022

Published:
31 August 2022

ABSTRACT

Vine was reintroduced to the volcanic island of Lanzarote (Spain) more than 300 years ago. The phylloxera plague has never reached the island; consequently, *Vitis vinifera* L. has evolved continuously since then due to the influence of some abiotic factors, such as low rainfall and strong winds. The aim of this study was to determine the inter- and intra-varietal diversity of the island's vines and the potential evolution of singularity. Two hundred and twenty-three samples were genotyped with 20 SSR (Simple Sequence Repeats). The following results were obtained from these analyses: 8 new varieties (Blanca de la granja del cabildo (W), Burra chinija (W), Diego chinija (W), Lemes del Cabezo (W), Malvasia alistanada fina (W), Malvasia alistanada rustica (W) (a mutation of Malvasia alistanada fina), Sinforiano chano (Rs), Uvillón negro (R), Vijariego blanco de la granja (W)), and 2 new sports (Chasselas lajiares (Rs) and Diego de El Raso rosado (Rs)). In addition, scientific studies on varieties from the island of Lanzarote describe four local cultivars: Breval negro (Rg), Malvasia volcanica (W), Torrontes volcanico (W) and Uva de año (R), which means that a total of 12 local varieties and 2 colour mutations have been described for this Atlantic island. It should also be noted that the appearance of 100 new molecular profiles with their corresponding names represents an unprecedented lexical enrichment. Lanzarote can be considered a centre of biodiversity, as is the Canary archipelago as a whole. Their vines have evolved to such an extent that they are now genetically different from the original populations. The vines from Lanzarote could be of great interest to the wine sector, as they can act as a nursery from which better-adapted vines can be obtained to mitigate the effects of climate change.

KEYWORDS: Canarian vines, Spanish varieties, local variety, SSR, microsatellite

INTRODUCTION

The varietal diversity of *Vitis vinifera* L. is considerable (This *et al.,* 2006), with current widespread varieties, other old and not so common varieties and even those that have parental relationships with wild vine (Ocete *et al.,* 2019). This biodiversity has also increased with the appearance of interspecific hybrids that can adapt to cold climates or resist certain diseases (De Oliveira *et al.,* 2020). This *et al.* (2006) reported the existence of more than 5,000 different cultivars of *Vitis vinifera* L. (information based on molecular DNA profiles). In their paper, Wolkovich *et al.* (2018) state that only twelve cultivars account for between 70 and 90 % of the world's vineyard surface (Cabernet-Sauvignon, Chardonnay, Merlot, Pinot noir, Syrah, Sauvignon blanc, Riesling, Muscat a petits grains blancs, Gewürztraminer, Viognier, Pinot blanc and Pinot gris). This shows that only a very low percentage of the total diversity is preferentially used, probably due to anthropogenic selective pressure. Consumer preferences and certain global laws, and their adaptation to different environments throughout the world, are probably the main reasons why these so-called "international" varieties are used to the detriment of local or minority varieties.

According to FAO (Food and Agriculture Organization of the United Nations), (http://faostat.fao.org) vines for winemaking are the most economically valuable crop (Stanimirovic *et al.,* 2018). Their value is closely linked to "terroir" (Wolkovich *et al.,* 2018), a concept that includes factors such as soil, climate, landscape, management of viticultural exploitation, product management and winemaking, as well as cultural and socioeconomic factors (van Leeuwen and Seguin, 2006). Of these factors, location and climate seem to be the most important (Jones, 2003; Gladstones, 2011).

The scenarios envisaged by the Intergovernmental Panel for Climate Change (IPCC, 2014) forecast changes in rainfall and temperature regimes for the coming decades and higher frequencies of droughts and extreme temperatures (Sancho-Galán *et al.,* 2020). These changes in local climate represent a great challenge, since they will probably decrease yield production and wine quality (Lobell *et al.,* 2011). Given this situation, the high diversity of *Vitis vinifera* L. cultivars may be very useful. Therefore, Lipper *et al.* (2014) suggest exploring the inter- and intra-varietal diversity of *Vitis vinifera* L. as a possible strategy for increasing its potential for adapting to the changing climate conditions, which will require resistance and sustainability (Jimenez-Cantizano *et al.,* 2020).

In short, the flexibility of the *Vitis vinifera* L. species is being increasingly studied so that grape production can be adapted to climate change, with particular focus on its hidden diversity (Sehgal *et al.,* 2015; Jimenez-Cantizano *et al.,* 2018; Jimenez *et al.,* 2019; Yuste *et al.,* 2019). In addition, studies on different crop types, including the grapevine, usually find that local varieties are superior to the more widely planted varieties in terms of resistance to abiotic stress (preferably water stress) and diseases (Newton *et al.,* 2010; Vikram *et al.,* 2016).

The Canary Islands (Spain) are located south-west of Spain and north-west of Africa off the coast of Morocco. Due to its strategic position in the Atlantic Ocean, it was an almost obligatory stopover for navigation between Europe, Africa and America (Figure 1).

The archipelago is part of the area known as Macaronesia, a group of five volcanic archipelagos (the Azores, Canary Islands, Cape Verde, Madeira and the Savage Islands) near the African continent (Santamarta and Naranjo Borges, 2015; Pérez *et al.,* 2020). Spain and Portugal colonised these islands in the fifteenth century; therefore, the introduction of cultivated vine can be mainly attributed to the colonisers from these countries (Macias, 2005). One of the most relevant historical events within the wine sector is that, except for Madeira, phylloxera never attacked the other islands of these five archipelagos. This has enabled the asexual reproduction system to be used to propagate the vine for five centuries using cutting or layering. Many individual varieties of *Vitis vinifera* L. currently in these archipelagos are therefore the result of 1) natural selection as an adaptation to new habitats, 2) natural crosses, 3) anthropogenic selection, and 4) mutations (Forni, 2012). All of this suggests that this may be a centre of biodiversity (Marsal *et al.,* 2019).

Lanzarote is one of the two easternmost islands of the Canary archipelago (Figure 1) and, therefore, also one of the most arid (along with Fuerteventura). Night temperatures are rarely below 13 °C in winter and 20 °C in summer. The proximity to the tropics means that the average temperatures (22 °C) are not low enough for natural vegetative plant development to stop; therefore, it is necessary to induce it to stop by pruning. The low rainfall (less than 150 mm per year

FIGURE 1. The geographical location of the Macaronesian region (left) and Lanzarote (Canary Archipelago) (right).

on average) makes it necessary to optimise water resources. Lanzarote winegrowers know how to take advantage of the volcanic origin of the land. The lapilli layer in some areas can be up to 3 m thick and can be used for storing water. Likewise, the strong trade winds tend to dehydrate the plant and put it in danger (DOP Vinos de Lanzarote, 2019). All these factors mean that the vineyards are subject to extreme conditions and require constant manual care and maintenance. The viticulture, therefore, can be considered heroic (Figure 2).

Under these conditions, the vine species planted by the colonisers have undergone a process of adaptation to this extremely arid habitat over three hundred years. Although the cultivated vine had already been introduced to Lanzarote - as in the rest of the archipelago - in the middle of the 16th century, it had to be reintroduced after the volcanic eruptions of the Timanfaya volcano in 1736. These eruptions covered a third of the island with ash ('lapilli' or 'picón' in the local language), burying huge areas of fertile land. For this reason, its current vines descend from individual vines imported mainly from Spain and Portugal in the 18th century (directly or via neighbouring islands and archipelagos) (Macias 2005) and form a landscape of unique vineyards, buried in funnel-shaped holes or protected from winds by semicircular stone walls (Figure 2).

According to Ibañez *et al.* (2003) and Marsal *et al.* (2019), the island has four local varieties: Breval negro (rouge (Rg)), Malvasia volcanica (White (W)), Uva de año (Tinta (R)) and Torrontes volcanico (W). The parents of the Malvasia volcanica have been identified using 20 SSR (Simple Sequence Repeats) or microsatellites and SNPs (Zerolo *et al.,* 2006; Rodriguez-Torres, 2018). These are the canarian variety Bermejuela and Malvasia Dubrovacka (known in the Canary Islands as Malvasia aromatica). This latter variety is widespread throughout the archipelago and has no specific origin according to VIVC (*Vitis* International Variety Catalogue) (Maul and Röckel, 2015). Microsatellites or SSRs are the most widely used molecular markers and are available in several databases. They have proved to be very efficient for evaluating both genetic diversity and population structure, and are very suitable for constructing core collections (Emanuelli *et al.,* 2013; Cano *et al.,* 2021).

The main aim of this study was to carry out an exhaustive sampling in Lanzarote to obtain the most reliable record possible of the varietal distribution of grapevine on the island today. Another objective was to determine if the individuals with new genotypes originated as a result of the evolution of the vine, which had to inexorably adapt to a habitat characterised by volcanic soil and extreme climatic conditions (high temperature and very little rainfall). The additional specific objective was to find new synonyms, homonyms and errors, and highlight the singularity of the individuals cultivated in Lanzarote compared to those of the rest of the archipelago, the Iberian Peninsula and the world.

MATERIAL AND METHODS

1. Plant material

Two hundred and twenty-three mature vine shoots (*Vitis vinifera* L.) were collected in Lanzarote using mass selection based on information from the winegrowers. These vineyards are planted with different varieties according to the traditional customs of the island. Consequently, these vineyards are not single-cultivar. Once collected, they were conserved at -20 °C until they were processed. Four well-characterised cultivars were also included as control samples (Marsal *et al.,* 2011): Chardonnay blanc, Garnacha blanca, Tempranillo tinto and Cabernet-Sauvignon cultivar plants from the Rovira i Virgili University experimental vineyard in Constantí (AOC Tarragona, Spain; 41°9′16.04″ (N) and 1°11′1.28″ (E)). Supplementary Table S1 shows detailed information about all the accessions and all other necessary information.

2. Microsatellite genotyping

DNA was extracted using the method described by Marsal *et al.* (2011) (based on Fort *et al*. (2008) protocol). The grapevine samples were genotyped using 20 SSR markers, which were selected for their capacity for discrimination and polymorphism in agreement with previous studies: VVS2, VVS3, VVS29 (Thomas and Scott, 1993); VVMD5, VVMD6, VVMD7 (Bowers *et al.,* 1996); VVMD27, VVMD28, VVMD36 (Bowers *et al.,* 1999b);

FIGURE 2. Panoramic view of a La Geria vineyard (Lanzarote).

VrZAG21, VrZAG47, VrZAG62, VrZAG64, VrZAG79, VrZAG83 (Sefc *et al.,* 1999); scu06vv (Scott *et al.,* 2000b); VvUCH11, VvUCH12, VvUCH19 (Lefort *et al.,* 2002); VChr19a (Cipriani *et al.,* 2010). These 20 SSR are not independent *loci*, because the VrZAG47 and VVMD27 describe the same microsatellite zone with different primer designs (Dalbó *et al.,* 2000). The international scientific community (This *et al.,* 2004; Maul and Röckel, 2015) uses seven of these as reference genetic markers.

Microsatellite amplifications were performed using Polymerase Chain Reaction (PCR) analysis and a MyCycler thermocycler (BioRad Laboratories, Hercules, California, USA). PCR was carried out with 50 ng of DNA and 1 µM of each primer with an attached fluorescent dye in the upper primer (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, VvUCH12) using the AmpliTaq DNA Polymerase kit (Applied Biosystems, Foster City, CA). The SSRs were divided into three groups according to the different thermocyclation required conditions based on their annealing temperature (Ta) (50 °C, 52 °C, and 58 °C). The programme was 95 °C for 5 min, 40 cycles (95 °C for 45 sec; Ta for 30 sec; 72 °C for 1 min 30 sec), and 72 °C for 7 min. The amplification products were mixed with 20 µL of deionised formamide and 0.25 µL of DNA size standard (GeneScan 500-ROX, Applied Biosystems, Foster City, CA), and denatured at 95 °C for 5 min (Marsal *et al.,* 2011; Marsal *et al.,* 2013). The fragments were separated by capillary electrophoresis with an ABI PRISM 3730® Genetic Analyser (Applied Biosystems, Foster City, CA). Peak Scanner Software (Applied Biosystems, New Jersey, USA) was used to size the amplified fragments. Each cultivar was analysed twice to prevent possible errors.

3. Data analysis

GenAlEx 6.5 software (Peakall and Smouse, 2012) was used to estimate the four genetic parameters: number of different alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He). The probability of identity (PI) and the estimated frequency of null alleles (*r*) was calculated using Identity 1.0 software (Wagner and Sefc, 1999). To distinguish homozygotes and heterozygotes for each locus, the data were considered codominant for data analysis.

Population structure and identification of admixed individuals were performed using the model-based software programme Structure 2.3 (Pritchard *et al.,* 2000; Falush *et al.,* 2003), which uses a model-based Bayesian clustering method. In this model, several populations (K) are assumed to be present, and they are each characterised by a set of allele frequencies at every locus. Individuals in the sample are assigned to populations (clusters), or jointly to more populations if their genotypes indicate that they are admixed. All *loci* are assumed independent, and each K population is assumed to follow the Hardy-Weinberg equilibrium. The subsequent probabilities were estimated using the Markov Chain Monte Carlo (MCMC) method. The 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 15, 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 described by Evanno *et al*. (2005). ΔK is a quantity based on the rate of change in the log probability of the data between successive K values. The Structure 2.3 programme was also used to create a phylogenetic tree to establish the relationships among populations. For this purpose, a pairwise matrix of their genetic distances was used according to the method reported by Nei (1972). This matrix was subsequently used to generate a phylogenetic tree based on the Neighbor-Joining method (Saitou and Nei, 1987).

The frequency-based assignment test (Paetkau *et al.,* 1995; Paetkau *et al.,* 2004), also available in GenAlEx 6.5, was first used to assign the accessions to each subpopulation generated by Structure. For each accession, a log-likelihood value was calculated for each subpopulation using the allele frequencies of the respective subpopulations. An individual was assigned to the subpopulation with the highest log-likelihood value. In addition, this software was also used to calculate genetic differentiation through the analysis of molecular variance (AMOVA) with 999 permutations of the data set for SSR genotypes with Rst assuming a gradual mutation model, and Fst assuming the model of infinite alleles. Finally, Principal Coordinate Analysis (PCoA) in GenAlEx 6.5 was used to further examine the genetic relationships between subpopulations based on the same SSR data. PCoA was based on the standardised covariance of the genetic distances calculated for co-dominant markers.

RESULTS

The 223 individuals from the prospection of Lanzarote were molecularly characterised and identified using 20 SSR. The molecular SSR profiles (MP-SSR) were compared with genetic reference profiles (Marsal *et al.,* 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019).

1. SSR Polymorphism

The same set of 20 SSRs (Supplementary Table S2) was used to characterise a population sample as in previous studies by Marsal *et al.,* 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019). Ninety-nine unique MP-SSRs were obtained; these included profiles of individuals from the main variety with variations (mutations) and excluding sports. Under these conditions, two hundred and twenty different alleles (Na) were counted. The average number of alleles per locus was 11 (3 (VVS3) - 24 (VVMD28)). The Ne with an average value of 4.4 ranged between 1.4 (VVS29) and 9.5 (VVMD28). The mean values

found for Ho and He were 0.741 and 0.700 respectively, while the probability of null alleles (r) fluctuated between -0.1314 (VVS3) and 0.1155 (VVMD6). Finally, the accumulated PI had values of 1.3x10-20 ranging from 0.020 (VVMD28) to 0.546 (VVS29).

2. Cultivar analysis

The objective of this analysis was to confirm whether the name of each accession coincides with that of an existing variety and, in turn, if the MP-SSR also coincides. Considering the great diversity of both names and MP-SSR for the different grape varieties, an exhaustive bibliographic study was made of all the information collated in databases, scientific articles, reviews and/or books about Canarian varieties (*Vitis* Canarias, 2015; Marsal *et al.,* 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019; Zerolo *et al.,* 2006; Rodriguez-Torres, 2018). This information was verified in the VIVC when the name and/or the MP-SSR were not found.

Supplementary Table S1 shows the original information of the 223 accessions that make up the population of the Lanzarote prospection (original code, laboratory code, accession name, berry colour, use and plot of land). The conclusive information for each accession, the % similarity of the nearest genotype, and the alleles that mutate are also shown. Supplementary Table S3 displays 99 unique MP-SSRs, which means that 123 MP-SSRs were rejected, since their MP-SSRs were identical to those of other individuals. The only exception shown in this table was the inclusion of accession 128LZ, which coincided with the MP-SSR of accession 106LZ, but differed from it in name and colour (sport). In addition, this table S3 shows the 7 international SSRs (This *et al.,* 2004) for the 99 unique MP-SSRs and the sport. This table also contains information about the proximity of each mutation to the main genome described in the TECNENOL database (Marsal *et al.,* 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019), and it gives details of the mutated and triallelic alleles. It is important to highlight that the individuals that make up both tables correspond to 33 different varieties, of which 25 are published and 8 are new varieties. Of the 25 known varieties, 4 are French (Alicante Henri Bouchet, Alphonse Lavaillee, Cabernet-Sauvignon and Syrah), 1 has a Central Europe unspecified origin (Chasselas blanc), 2 are Greek (Muscat a petits grains blancs and Muscat of Alexandria), 4 are Portuguese (Malvasia fina, Tinto cao, Touriga nacional and Verdelho branco), 9 are peninsular Spanish (Airen, Beba, De rey (Sancho-Galán *et al*., 2019), Ferral, Garnacha tinta, Lisan prieto, Mollar cano, Palomino fino (Sancho-Galán *et al*., 2021) and Vijariego blanco), 2 are local varieties from the Canary Islands (Bermejuela and Listan vegro) and the remaining 3 correspond to local varieties from Lanzarote (Breval negro, Malvasia volcanica and Torrontes volcanico).

Supplementary Table S3 shows that 16 MP-SSRs are identical to MP-SSRs of the main variety (thus we will refer to them as "Identity"), as well as 8 new MP-SSRs (varieties). Regarding the 75 MP-SSRs that present variations in the alleles of the SSRs studied, 30 MP-SSRs have a single variation in their MP-SSR (mutation-97.5 %), 28 MP-SSRs contain two variations (mutation-95 %), 10 MP-SSRs show 3 variations (mutation-92.5 %), 4 MP-SSRs have 4 mutations (mutation-90 %) and finally 3 MP-SSRs show five variations. To summarise, 4 % of the alleles possessed by the known varieties show some variation, either spot or trialelism (0.25 %). Supplementary Table 3 also shows 3 accessions that correspond to colour variations (sport), Beba roja that in this study represents an identity included in the VIVC, and two new colour mutations, Chasselas lajiares and Diego de El Raso rosado (sport of the Diego de El Raso mutation (106LZ)). Finally, it should be noted that the 75 MP-SSR that show variations in this population correspond to 19 varieties, which means that there are 14 varieties with identical individuals to the main MP-SSR (6 known varieties and the 8 new local varieties from Lanzarote).

Thirty-two MP-SSRs from Supplementary Table S3 correspond to the 3 local varieties of Lanzarote. The Breval negro variety was registered in this collection under the name Perejil tinto, a specimen that turned out to be an Identity. The Malvasia volcanica variety is composed of 29 MP-SSRs, of which 8 only showed one mutated allele, 11 showed 2 alleles with variations, 6 showed three mutated alleles, 1 sample showed 4 variations in its alleles, and finally another MP-SSR showed 5 variations. The last variety from Lanzarote is the Torrontes volcanico that contains 2 MP-SSRs, showing 2 and 4 variations respectively, with the presence of a specimen with a case of trialelism.

The results of the correspondence analysis between the names of the 223 accessions registered with the MP-SSR of the main variety detected (lexical study) are shown in Supplementary Table S1. It should be noted that 163 correct names were detected (88 fully coincided with the main name, and the remaining 75 contained lexical variations), in addition, 11 names were found that corresponded to registered synonyms of the main variety. We also detected 12 names of samples that were registered under a synonym or homonym name of another variety, and 24 names were errors (confusion or ignorance). Two accessions were littered as unknown, and two other samples, which were fully identified, had a new name synonym. The nine new MP-SSRs had new names (one MP-SSR corresponded to a variation of a new variety).

3. Genetic structure of the Lanzarote population

The study of the genetic structure of the Lanzarote population was based on the population of 99 unique PM-SSRs. To avoid false results, the data was normalised. Thus, all redundant information was removed (66 unique MP-SSR). Mutants, sport, and the new PM-SSR Malvasia alistanada rustica (very closely related with the cultivar Malvasia alistanada fina) were excluded. However, the selected individuals are not always standard profiles, because in some cases there was only one mutated representative of the identity considered by the databases. This was the case of the MP-SSRs of the varieties Cabernet-Sauvignon, De rey (Sancho-Galán *et al*., 2019), Garnacha tinta, Malvasia fina, Mollar cano, Muscat a petits grains blancs, Muscat of Alexandria, Torrontes

volcanico and Touriga nacional, which in this Lanzarote population are represented by slightly mutated individuals. Hence, the study of the genetic structure of the Lanzarote population focused exclusively on the 33 non-redundant PM-SSRs (25 know varieties and 8 new local PM-SSRs). This population contained 11 local PM-SSRs from Lanzarote (Breval negro, Malvasia volcanica, Torrontes volcanico, which is only represented by its mutation-95 % (Torrontes volcánico de melo), and the eight new MP-SSRs (Blanca de la granja del cabildo (W), Burra chinija (W), Diego chinija (W), Lemes de el Cabezo (W), Malvasia alistanada fina (W), Sinforiano chano (Rs: rosada), Uvillón negro (R) and Vijariego blanco de la granja (W)).

The population structure was analysed using Structure 2.3. software, with which the different distributions in our population (K) were determined. Supplementary Figure S1 shows how the best K was calculated according to the Evanno model was applied (2005). For our population, it was K = 5, which means that all the varieties can be clustered into five ancestral groups. Figure 3 shows this distribution for the 33 representatives (MP-SSR), which are both pure and admixed MP-SSRs. The varieties of these five groups were ordered according to their *q* value (the percentage of their inferred genome belonging to the group (Bacilieri *et al.,* 2013) (from highest to lowest)). This strategy allowed admixed varieties - which would have distorted the results of the study - to be excluded. Thus, in population POP1, three individuals were rejected (Alphonse de Lavallee, Torrontes volcanico de Melo (M-95 %) and De rey chicharrero-97.5 %), while in population POP3 only one was excluded (Bermejuela). In population POP4, four individuals were excluded (Listan prieto, Burra chinija, Beba roja and Malvasia fina de frasco-92.5 %), while POP2 and POP5 remained intact, since they both contained only pure MP-SSRs. This meant dispensing with eight individuals, of which one (Burra chinija) was a new MP-SSR (admixed with $q < 85$ %). As a result, the population of pure individuals had only 25 representatives ($q \geq 85\%$). The corresponding assignment test gave a goodness of fit of 100 % (data not shown).

Population POP1 consisted of 9 individuals of which four were standard varieties, four were mutations and one was a new Lanzarote local cultivar. Mostly foreign representatives (not from the Canary Islands) characterised this group. The exception was a new local variety (Blanca de la granja del cabildo). This group consisted of 44.44 % of white varieties and 55.55 % of red. In addition, 66.66 % of the individuals of this group are typically used for winemaking, while 22.22 % are used as table grapes and in winemaking, and the remaining 11.11 % are used for table grapes, winemaking and raisins. POP2 consisted of 3 individuals, 66.66 % of which were local Lanzarote varieties (Vijariego blanco de la granja and Diego chinija). The only exception was Vijariego blanco, a Spanish variety from Andalusia. All the varieties in this group were white, 66.66 % of which are used for table grapes and 33.33 % for winemaking. The third subpopulation (POP3) comprised 4 varieties, all from Lanzarote: the local variety Malvasia volcanica, and three new varieties, Malvasia alistanada fina, Sinforiano chano and Lemes del cabezo. This group comprised 75 % of

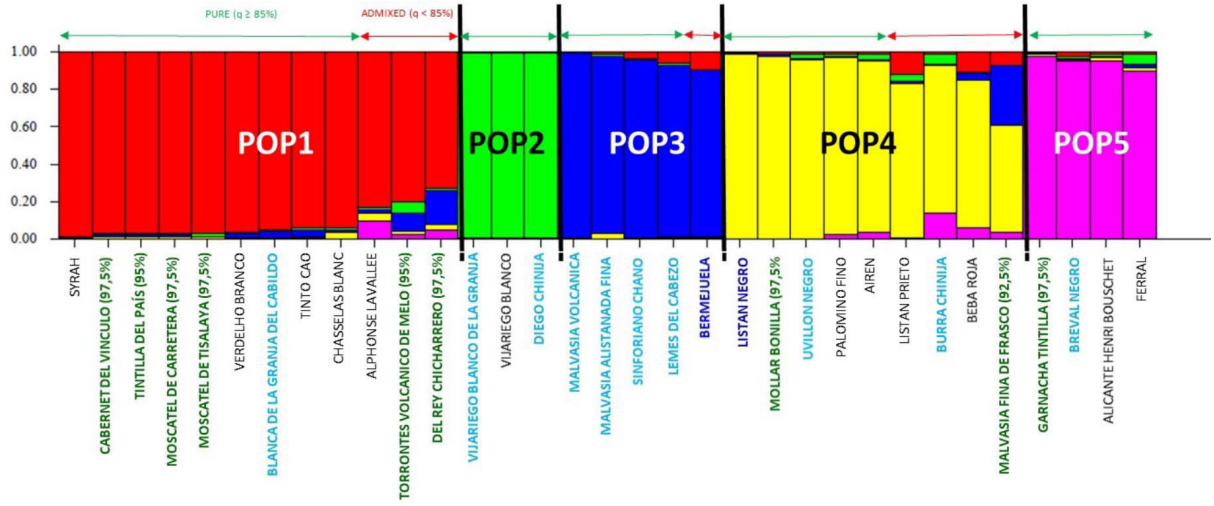

FIGURE 3. Structure graph of 33 individuals from Lanzarote collection, when the population is divided into 5 groups (5K).

Prime name: Prime name according to VIVC (Maul and Röckel, 2015).

Prime name: (in bold type): Local variety from the Canary archipelago.

Prime name: (in bold type): Variety proposed as local from Lanzarote or local variety from Lanzarote.

Mutation name: Individual that corresponds to a mutation of a variety. The percentage of similarity regarding the main genetic profile is indicated.

white varieties and 25 % of rouge (Rs). In addition, 75 % of the grapes in this group is typically used for winemaking, while 25 % is used as table grapes and for winemaking. Subpopulation POP4 contained five varieties, one of which was new (Uvillon negro) and can be considered to be a local Lanzarote variety. The other cultivars were the standard Spanish varieties Palomino fino (Sancho-Galán *et al*., 2021), Airen, Listan negro (a local canary cultivar) and a mutation of Mollar cano (Mollar bonilla-97.5 %). Forty percent comprises white grapes, whereas 60 % comprises black grapes. These cultivars are mostly used for both purposes, table grapes and winemaking, except for Listan negro which is only used for winemaking. Finally, the last group has 4 black (or rouge) varieties, of which three were standard - Breval negro (rouge and LNZ (Lanzarote)), Alicante Henri Bouchet (FRA) and Ferral (ESP) - and one was the Spanish mutation, Garnacha tintilla (97.5 %). Two of these varieties are for winemaking, one is for table grapes and the other is for both purposes.

The results of the principal coordinates analysis showing this distribution are presented in Figures 4 and 5. On the left of Figure 4, it can be observed that coordinate 1 (with a goodness of fit of 24.73 %) separates POP1 from the rest (POP2, POP3, POP4, and POP5). These subpopulations are located in the quadrants on the left and are formed by Spanish individuals with the only exception being POP5, which contains a French cultivar (Figure 5, at the top). Coordinate 2 (41.63 %) practically isolates POP5 in the upper quadrants, leaving POP1 on the very far right-hand side. The rest of the subpopulations (POP2, POP3, and POP4), are located in the lower hand quadrant. The distribution of the individuals (Figure 5 at the top) shows similar behaviour with adequate percentages of goodness of fit. Phylogenetic trees of the Lanzarote cultivar population are shown on the right hand side of Figure 4 and at the bottom of Figure 5 show. This data shows that there are 3 branches containing: i) POP5, ii) subpopulations of POP1 and POP3 subpopulations, and iii) POP2 and POP4.

4. Genetic structure of the Lanzarote population in a world population

The objective of this section is to demonstrate the uniqueness of the MP-SSRs that make up the population of local cultivars in Lanzarote, whether they correspond to varieties that have already been published or to new MP-SSRs from this study. Already published varieties comprised Brebal negro Malvasia volcanica, Torrontes volcanico and Uva de año (Ibañez *et al.,* 2003; Marsal *et al.,* 2019). MP-SSRs not previously published and presented for the first time in this study as being new comprised 8 new local MP-SSRs: Blanca de la granja del cabildo, Burra chinija, Diego chinija, Lemes del Cabezo, Malvasia alistanada fina, Malvasia alistanada rustica (which was excluded, because it is a mutation of Malvasia alistanada fina), Sinforiano chano, Uvillón negro and Vijariego blanco de la granja). Therefore, there was a population of local MP-SSRs from Lanzarote composed of 12 varieties. The world population comprised cultivars from the TECNENOL database (Marsal *et al.,* 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019) and all of them were analysed with the same 20 SSRs. Of the 290 MP-SSRs (dismissing author crossings and interspecific hybridizations), 11 corresponded to Canarian varieties (Marsal *et al.,* 2019).

The method used was the same as that described in the section above. The Structure 2.3. software which was once again used gave a distribution value of K = 2 for a final population of 301 varieties (Supplementary Figure S2). Therefore, two subpopulations were formed (2K): POP1 and POP2 (Supplementary Table S4). Of the 182 components in subpopulation POP1, 162 were pure varieties ($q \geq 85$ %) and 20 admixed varieties ($q < 85$ %). The subpopulation POP2 was smaller and contained only 119 varieties comprising 96 pure and 23 admixed cultivars. In this case, a Canarian

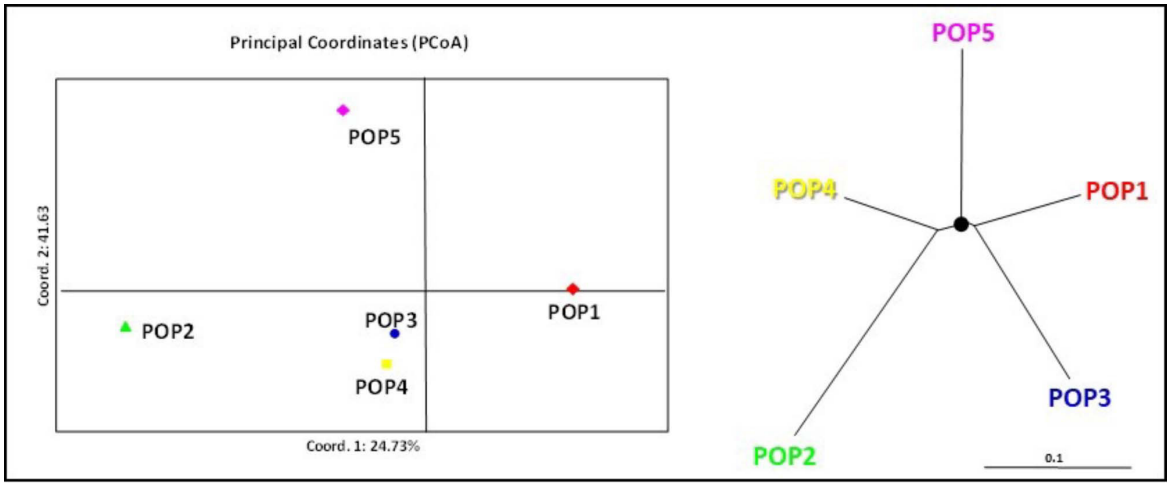

FIGURE 4. Lanzarote population when divided into 5 groups (5K). Left: Principal Coordinate plot of Lanzarote populations. Right: Phylogenetic tree of Lanzarote populations.

In order to ensure a reliable characterisation of each group, only the varieties with $q \geq 85$ % were taken into account.

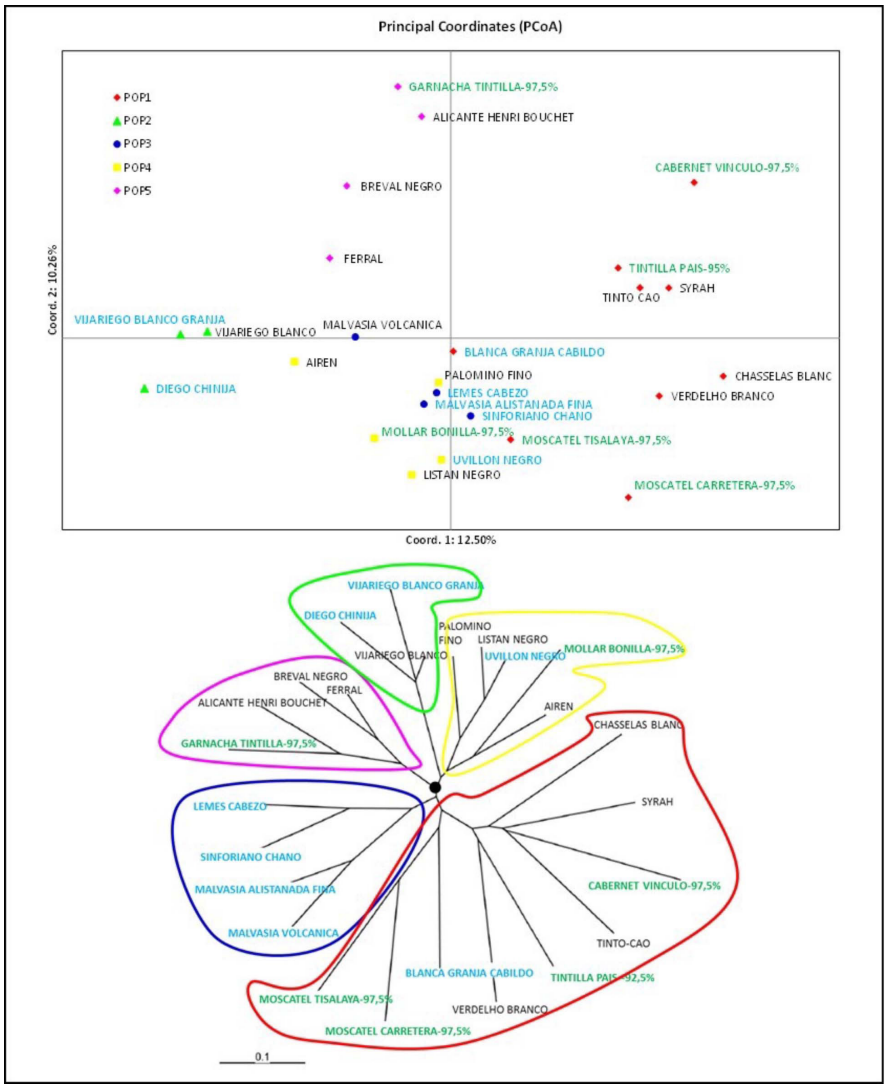

FIGURE 5. Lanzarote population divided into 5 groups (5K). At the top: Principal Coordinates Plot of individuals from Lanzarote population. At the bottom: Phylogenetic tree of Lanzarote individuals.

Prime name: Prime name according to VIVC (Maul and Röckel, 2015); Prime name: (in bold type): Variety proposed as being local from Lanzarote; Mutation name: Individual that corresponds to a mutation of a variety. The percentage of similarity regarding the main genetic profile is indicated.

In order to ensure a reliable characterisation of each group, only the varieties with q ≥ 85 % were taken into account.

variety was found to be an admixed variety (Albillo criollo). The next step was to remove the admixed individuals, which left a total population of 258 individuals distributed among 162 varieties in POP1 and 96 varieties in POP2. An assignment test was performed under these new conditions, which gave a goodness of fit of 100 % (data not shown). The POP2 subpopulation was mainly composed of individuals from the Iberian Peninsula, while the varieties that were mainly part of POP1 were from the rest of the world. To demonstrate the uniqueness of both the population of Canarian varieties and the population of Lanzarote varieties, the 10 local Canarian MP-SSRs (IC) and the 12 local Lanzarote MP-SSRs (LNZ) were extracted from the POP2 subpopulation to which they belonged. In this way, POP2 was reduced to 22 components. Using this new distribution, the assignment test had a goodness of fit of 93 % (data not shown). Figure 6a (left) shows the distribution of these four subpopulations in a principal coordinate plot. With a goodness of fit of 53.79 %, coordinate 1 separates the Canary Island populations (quadrants on the left) from those of the rest of the world (quadrants on the right). In contrast, coordinate 2 (34.22 %) separates the populations that are strongly influenced by the Iberian Peninsula (IP) from the others. In Figure 6a (left) each of the four subpopulations is in a different quadrant, LNZ being almost equidistant. Thus, the local Lanzarote varieties are shown to be unique, since they are much further from the subpopulation POP2 (of which they were native) than from the Canarian varietals (IC). The phylogenetic tree (Figure 6a (right)), confirms the results obtained in principal coordinate plots. This figure clearly shows a separation into three large branches, POP1, POP2 and the Canarian subpopulation. In addition, this last subpopulation is divided, in turn, into

two island subpopulations (IC and LNZ). It can also be observed that the world subpopulations POP1 and LNZ are the furthest from the rest, since they have the longest ramifications, especially LNZ.

The Molecular Analysis of Variance (AMOVA) is a method used for estimating the differentiation between populations from molecular data. The AMOVA is used to calculate the Fst statistic - which takes into account the identity of alleles in the infinite allele model (IAM) - and the Rst statistic -which is derived from the allelic sizes in a stepwise mutation model (SMM). In the AMOVA test for the case of 2K (two clusters), IC and LNZ, it was found that the global genetic variation at the population level was high within each of these populations (96 %), while between them it was very low (4 %) (Summary Figure 3a). The Fst statistics and its analog the Rst statistic were also obtained. Both the Fst value (0.041) and the Rst value (0.023) for the world population were very low, and both results were significant at 0.001 and 0.01 confidence levels respectively (Summary Table S5a). In this context, when the populations are compared in pairs, Table S5a shows how the Lanzarote population is one of the furthest away according to the Fst statistic (0.052) and the furthest according to the Rst statistic (0.044) concerning the POP1 population. On the other hand, for the POP2 and IC populations, the lowest values were obtained for both Fst and Rst. These statistics present significant confidence levels (Cretazzo *et al*., 2022).

To confirm the uniqueness of the local Lanzarote varieties, we introduced the geographical component of the country of origin according to the VIVC (Maul and Röckel, 2015). As some populations have few representatives, it was decided to group the 301 varieties by geographic area according to the bibliography (Arroyo-Garcia *et al.,* 2006; Bacilieri *et al.,* 2013; Marsal *et al.,* 2017; Marsal *et al.,* 2019). The following five subpopulations were selected: subpopulation EASTMED-CAU (Algeria, Cyprus, Georgia, Israel, Lebanon, Tunisia and Turkey), subpopulation BP (Bosnia and Herzegovina, Bulgaria, Croatia, Greece, Serbia, Slovenia and Montenegro), subpopulation ITA (Italy), subpopulation FRA-CEU (Austria, France, Germany, Hungary and Switzerland) and subpopulation IP (Spain and Portugal), as well as subpopulations IC and LNZ. The assignment test gave a goodness of fit of 62 % (data not shown). The wrongly placed individuals were dismissed, as they were considered to be admixed. The world population was reduced to 260 individuals in 7 subpopulations and with a goodness of fit of 95 % (data not shown). The subpopulations of local Canary and Lanzarote varieties had the same representatives as described above. The distribution of these 7 subpopulations is shown in Figure 6b (left). Coordinate 1 (35.04 %) separates the Iberian populations from the others and places them in the quadrants on the left. Meanwhile, coordinate 2 (26.46 %) separates the EASTMED-CAU subpopulation (in the upper right hand quadrant) from the rest, leaving the IP, IC and LNZ subpopulations in the upper left hand quadrant, just at the limit of this coordinate. The BP, IT and FRA-CE subpopulations are located in the lower right quadrant. Figure 6b (right), which represents this geographical data in form of a phylogenetic tree, again

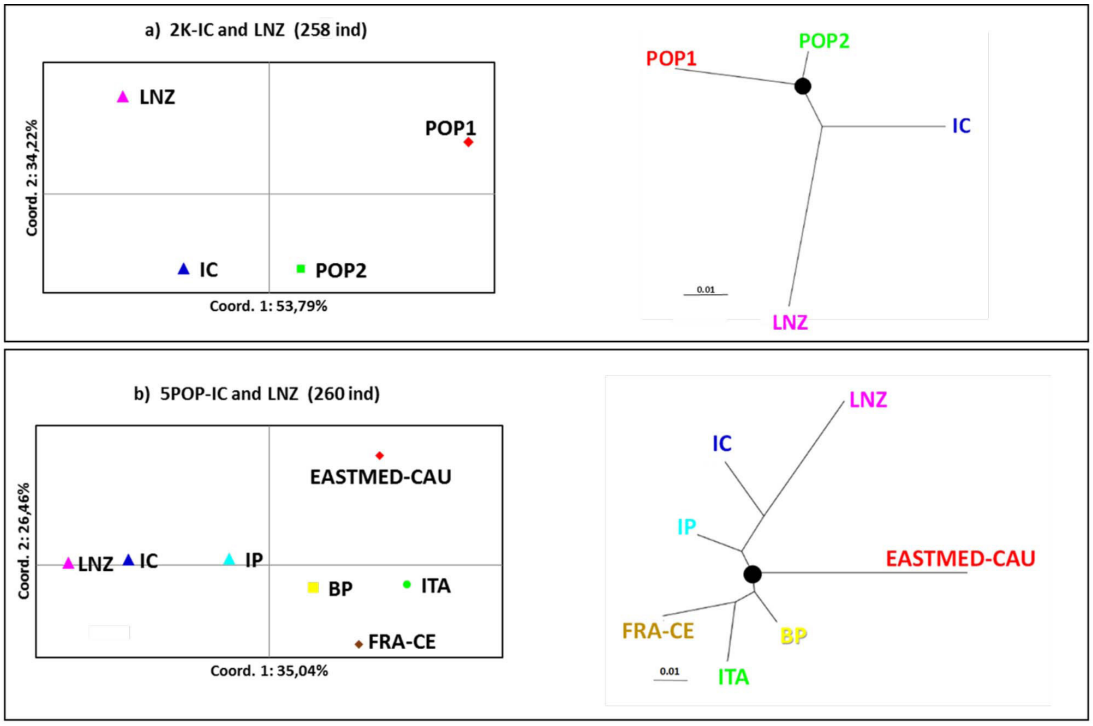

FIGURE 6. Principal Coordinates (left) and Phylogenetic Tree (right) of world populations: a) 2K-IC and LNZ population (POP1, POP2, IC, and LNZ), and b) 5 area-countries-IC and LNZ population.

In order to ensure a reliable characterisation of each group, only the varieties with q ≥ 85 % were taken into account.

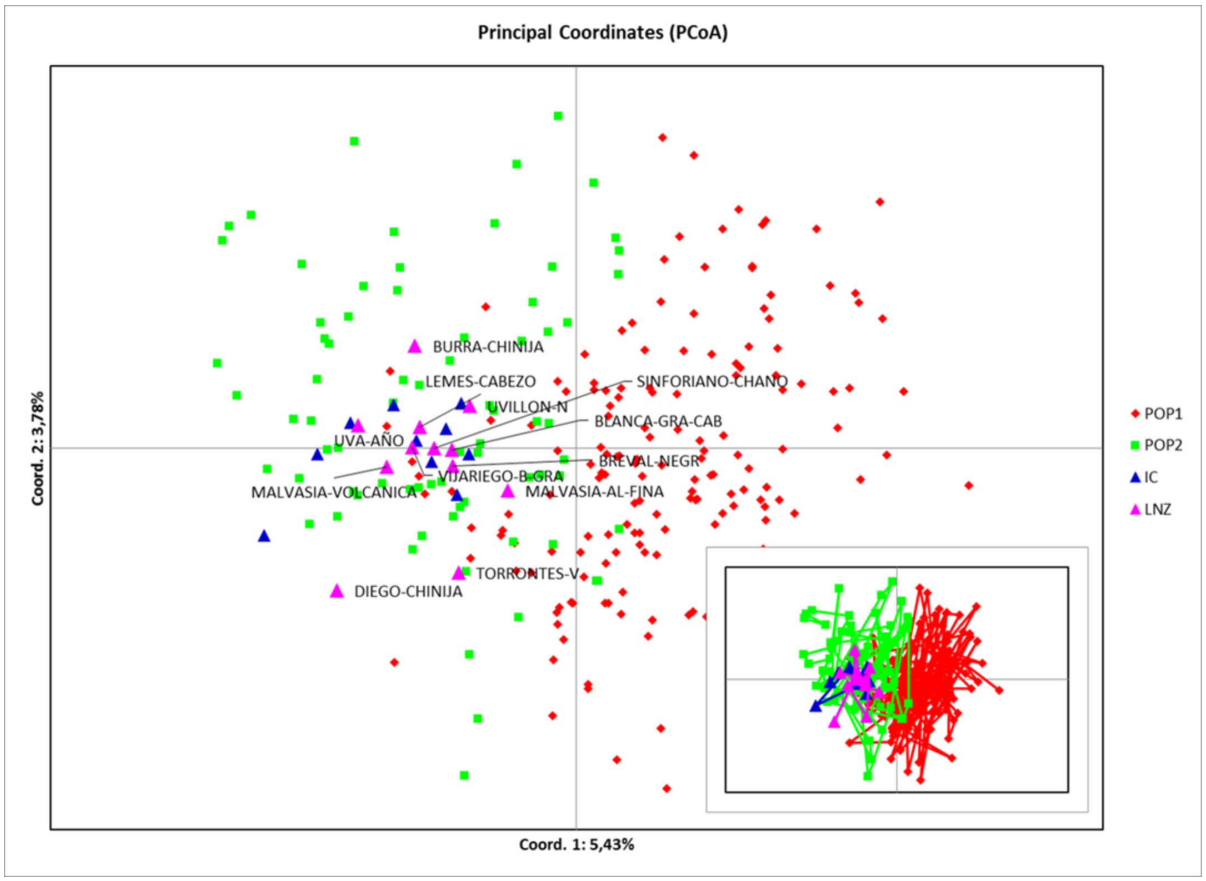

FIGURE 7. Principal Coordinate plot of individuals from the world population according to 2K + IC + LNZ subpopulations.

In order to ensure a reliable characterisation of each group, only the varieties with q ≥ 85 % were taken into account.

shows 3 highly differentiated branches; One corresponds to EASTMED-CAU, the second branch to the European subpopulations and the third to the IP subpopulations and the two Canary Island subpopulations. It should be noted that this last branch is subdivided into two branches: one contains IP and the other contains the IC and LNZ subpopulations separately. Figure 7 provides an overview of the distribution of local Lanzarote varieties among the cultivars in the world population. In this case, given a large number of varieties, coordinates 1 and 2 only explain 5.74 % and 3.92 % of the general variance for 2K + IC + LNZ. These values are quite low, but other studies have obtained similar results (Myles *et al.,* 2011; Emanuelli *et al.,* 2013; Augusto *et al.,* 2021). In any case, this figure shows how all the local varieties from LNZ are located on the left hand side of the graph, the majority being in the upper quadrant. The left hand side also contains some varieties of the IC and POP2 subpopulations.

In order to be able to study the genetic differentiation of the population of the island of Lanzarote, we analysed the data in the geographical distribution (5POP-IC and LNZ) by carrying out the AMOVA test. Supplementary Figure 3b shows how the overall genetic variation at the population level was again high within each of these populations (95 %), while among them it was very low (5 %), with percentage values being almost the same as those shown in Supplementary Figure 3b. The Fst and Rst values of are now almost the same, being 0.055 and 0.052 respectively, and both results were significant with a degree of confidence of 0.001. These results are consistent with those obtained by Cretazzo *et al*. (2022) (Summary Table S5b). Summary Table S5b shows that, in terms of its pairwise relationship with other world populations, the population of Lanzarote has the lowest Fst and Rst values for the PI and IC populations, both with good high levels of significance. The rest of the geographical areas show a greater genetic differentiation concerning the Lanzarote population, with the Balkans area being the closest.

DISCUSSION

The values obtained from the statistical characterisation of the 20 SSR used in this study (Supplementary Table S2) are within acceptable ranges according to most of the publications consulted. It is known that the results of such statistical analyses depend on the number of samples and the proximity between them, and the number of SSRs used

(Ibañez *et al.,* 2003; Lopes *et al.,* 2006; Milla-Tapia *et al.,* 2013; Stajner *et al.,* 2014; Marsal *et al.,* 2016; Aliquo *et al.,* 2017; Marsal *et al.,* 2017; Moita *et al.,* 2018; Marsal *et al.,* 2019). The exception is the gene diversity index (He) since its average is somewhat lower. It should also be noted that there is high variability in Ho (0.253 (VVS29) - 0.970 (VVMD5) and He (0.266 (VVS29) - 0.895 (VVMD28)). This behaviour was expected since many of the individuals studied turned out to be mutations of the same variety and consequently they have very close MP-SSR. It must also be pointed out that 16 markers had a probability of r lower than or equal to 0.01, which means that there was a very small proportion of excess heterozygosity. Two of the remaining markers had values somewhat higher than 0.01 (VVS2 and VVMD7), but in any case, they can be considered equally effective. This data confirms, therefore, that all the homozygous profiles obtained using these SSRs are true, and thus they would not have lost any alleles. In contrast, two other markers (VVMD6 and SCU6) had relatively high values (0.1126 and 0.1155 respectively) and therefore their discriminant utility can be considered as not so effective. The value of PI found in this population was somewhat higher than the results reported for Canarian varieties (Marsal *et al.,* 2019), but it was very similar to the values found in other studies (Stajner *et al.,* 2014). Therefore, it is possible to confirm that these 20 SSRs can distinguish between very close MP-SSRs. Furthermore, for this population, the SSR VVMD28 (Na: 24 / He: 0.895 / PI: 0.020 / r: -0.0183), VVMD36 (Na: 17 / He: 0.860 / PI: 0.033 / r: -0.0374), VVMD27 (Na: 14 / He: 0.874 / PI: 0.029 / r: -0.0403), VVMD5 (Na: 13 / He: 0.824 / PI: 0.0488 / r: -0.0709) and VVZAG47 (Na: 14 / He: 0.866 / PI: 0.032 / r : -0.0447) were the most informative microsatellites for identification purposes because of the large number of alleles found, the absence of null alleles and the low probability of identity (Ibañez *et al.,* 2003; Crespan, 2003b). The most limited microsatellites were the SSR VVS3 (Na: 3 / He: 0.464 / PI: 0.382 / r: -0.1314) and VVS29 (Na: 8 / He: 0.266 / PI: 0.546 / r: 0.0109). In general, these data confirm that this set of 20 SSRs is suitable for carrying out this molecular characterisation study.

The molecular profiles (MP-SSR) obtained in the present study were compared with reference genetic profiles (Marsal *et al.,* 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019): greater homogeneity in this population was observed compared to previous studies carried out by this group of authors, since all of the samples come from a single island. The analysis of intervarietal variability reaffirms this homogeneity, since 55 % of the 223 analysed accessions were rejected due to being redundant MP-SSRs. It would be from the remaining 99 MP-SSR when it would manifest that these would correspond to 25 known varieties and 8 new MP-SSR (33 MP-SSR). These results allowed us to highlight the intra-varietal variability of the island of Lanzarote. Thus, as can be seen in Summary Table S3, the Malvasía volcanica variety comprises 29 different MP-SSRs; i.e., the MP-SSR that corresponds to the most widespread MP-SSR and 28 variations of it. Ultimately, around 4 % of the studied alleles showed variations, confirming the existence of a short evolutionary process resulting from the inexorable adaptation of Lanzarote's vines over a period of more than 300 years. This resulted in the appearance of mutated individuals and new varieties (due to the accumulation of variations, natural crosses and both natural and anthropogenic selection). Cultivars with a similarity percentage lower than 87.5 % can be considered as different varieties. In contrast, individuals with a similarity percentage higher than or equal to 87.5 % are thought to be mutations of their closest cultivar according to criteria established by Marsal *et al*., 2016; Marsal *et al.,* 2017; Marsal *et al.,* 2019). Other authors have reported similar percentages: Ibañez *et al.,* (2003) (SSR (2 alleles / 26) 92 %)), Velez (2007) (SSR (2 alleles / 18) 89 %)) and Cabezas *et al.* (2011) (SNP, 90 %). Nine MP-SSRs did not match any other database profiles (< 87.5 % similarity). Consequently, they must be considered as new local varieties of Lanzarote, since they had an accession name (Supplementary Table S1 and S3). Another aspect to take into account is the variations in the population of MP-SSR from Lanzarote due to different allelic lengths and to the appearance of new alleles. Some tri-allelic profiles, which are indicative of chimerism, were observed across markers and genotypes. Grigoriou *et al*. (2021) reported that the amplification of multiple alleles per locus in one accession is possible. An example illustrating a case of a tri-allelic locus was found in the SSR VVS29, for the variety Chasselas blanc. In this study the accessions of Chasselas blanc (IDE), Chasselas rose (IDE) and Chasselas de El Raso (mutation-97.5 %), displayed values of 177/179 for this SSR. So, Chasselas perejilla (mutation-97.5 %), and Chasselas Lajiares (mutation-95 %) showed three alleles: 169/177/179. This tri-allelism was also observed in a previous study for the 51-IB sample of the Balearic Islands, known in the Balearic archipelago as Peu de rata (Marsal *et al.,* 2017); however, this information is not provided in this article.

Furthermore, new names from Lanzarote can be proposed for inclusion in the VIVC, due to the cultural richness of its people. These new names are: the 8 names of the new varieties, 2 new synonym names (brown), 12 existing synonym names for other varieties that are proposed to be included as new synonyms for the studied variety (pink), 2 new names for mutations of colour described for the first time (violet) and 76 new names to denominate the new mutations described in this study (75 for known varieties (green) and 1 for an unknown variety). Regarding the names of the new mutations, it is important to note that the ampelography section of the VIVC database gives proper and characteristic names for the mutant individuals of certain varieties; this is the case for the Pinot meunier accession, a Pinot noir mutation (mutation-90 % in the TECNENOL database), the Chasselas cioutat, a Chasselas blanc mutation (mutation-97.5 % in the TECNENOL database) and their sports.

To study the genetic structure of the vines cultivated on Lanzarote, we used the Structure 2.3 software to normalise the data for a second time, thereby eliminating the admixed individuals and grouping the pure individuals into population groups by genetic affinity. The determination of the different

distributions in our population (K) is shown in Figure S1. According to these results, the best distribution was the one proposed in our study (K = 5), slightly better than the proposal to divide the Lanzarote population into 2 ancestral groups (K = 2). When dividing our population of 33 non-redundant MP-SSRs into 5 ancestral groups (Figure 3), pure MP-SSRs ($q \geq 85\%$) could be differentiated from mestizos ($q < 85\ \%$). In this way, the normalised population for study was reduced to 25 MP-SSR. POP1 contained mostly foreign representatives (not from the Canary Islands). The exception was the observation of a new local variety in this group (Blanca de la granja del Cabildo), allowing us to hypothesise that is is of non-Canary origin. POP2 contains 2 new local Lanzarote varieties (related to the Andalusian variety Vijariego Blanco) and POP3 contains 3 new individuals (linked to the local Lanzarote variety, Malvasia volcanica, or its parent, Malvasia Dubrovacka). POP4 (containing varieties from the southern half of Spain) and POP5 (comprising cultivars from the northern half) contains a local variety from Lanzarote. In all these cases, it can be hypothesised that either these new varieties are the result of a genetic drift due to the variations resulting from their adaptation to the abiotic conditions of the island, or they are the result of natural crosses, without forgetting the influence of natural or anthropogenic selection. The whole of Figure 4 shows how the populations of cultivated varieties from Lanzarote with little peninsular influence are always very close (POP1 and POP3 (the influence is due to the presence of individuals close to the original Malvasia Dubrovacka from the eastern Mediterranean)); however, the rest of the populations have a marked peninsular origin, either from the south (POP2 and POP4) or from the north (POP5). This fact is reinforced by the historical evidence published by Macías (2005), which describes both the military colonisation of the Spanish Crown of Castile-Aragon and the migration of settlers from Madeira and Azores (Portuguese colonies), which have been described in Lanzarote.

Very similar results were obtained in the analysis of the genetic structure of the cultivated grapevine population on Lanzarote concerning the grapevine population in the world. The results shown in Figure 6 indicate that the genomes of the local Lanzarote varieties are singular and are mainly influenced by the IP and slightly influenced by the Balkans. In this regard, the origin of the parents of the most emblematic variety of the Lanzarote should be remembered: it has been reported that Malvasia volcanica is a cross between Malvasia Dubrovacka (Malvasia aromatica), of unknown origin according to VIVC (but often said to originate from the eastern part of the Mediterranean Basin) and the local Canarian variety Bermejuela (Zerolo *et al.,* 2006; Rodriguez-Torres, 2018 and Marsal *et al.,* 2019).

The world populations of *Vitis vinifera* L. show a low and consistent genetic differentiation (Cretazzo *et al*., 2022). The results of this study agree with the low and significant values of Fst at the population level (less than 0.10 in practically all populations). These results show a close relationship between all the populations studied, which is to be expected due to the history of *Vitis vinifera* L.: Man, through artificial selection, has collaborated and still collaborates in its gene flow (Meirmans, 2012). Likewise, the Rst statistic showed even lower values, which in many cases were very close to zero or were even zero (panmixia), indicating and reinforcing the low genetic differentiation between populations. It should be noted that in this study the Rst statistic was used to reinforce or refute the Fst value due to its ability to reject the high and complex mutation rate that characterises microsatellites or SSR, and thereby avoid the overestimation of the genetic differentiation degree due to homoplasy (Balloux and Lugon, 2002). According to these results, we can confirm the singularity of the population of vines of Lanzarote, despite it being historically closely related to the Canary archipelago and the Iberian Peninsula, and despite the Balkans perhaps being of influence

It can therefore be concluded that a clear separation exists between the domestication or biodiversity centres of the Iberian Peninsula (and those influenced by it) from the rest (Arroyo-Garcia *et al.,* 2006; Cretazzo *et al*., 2022). It is worth noting that in Figure 7 all the populations related to the Iberian Peninsula and the Canary Islands are differentiated from the rest of the world populations, which are located on the right-hand side (POP1 subpopulation). This data shows once again the singularity of the peninsular domestication centre, as it was previously described by Forni (2012) and Arroyo-Garcia *et al.,* (2006) including the Canary Islands. A graph with a higher number of dimensions would probably allow us to demonstrate per individuals both the exceptionality of the Canarian subpopulations and the uniqueness of the Lanzarote subpopulation (remember Figure 6a (right- and left-hand sides). Like most islands, Lanzarote is home to a biological and cultural diversity that has originated from isolation and specific environmental conditions.

CONCLUSIONS

This study reports 8 new varieties (Blanca de la granja del cabildo (W), Burra chinija (W), Diego chinija (W), Lemes de el Cabezo (W), Malvasia alistanada fina (W), Malvasia alistanada rustica (W) (a mutation of Malvasia alistanada fina), Sinforiano chano (Rs), Uvillón negro (R) and Vijariego blanco de la granja (W)), and two new sports (Chasselas perejil rosado and Diego de El Raso Rosado) found on Lanzarote, probably as a result of the adaptation of specimens brought by colonisers in the 18th century. In addition, this study and other scientific studies about varieties from the island of Lanzarote describe 4 local cultivars: Breval negro (Rg), Malvasia volcanica (W), Torrontes volcanico (W) and Uva de año (R). Therefore, a total of 12 local varieties and 2 colour mutations have been described for this Atlantic island. This study also reports 75 individuals that are mutations of 19 varieties of this population. Given the novelty of this information, we suggest that their names and their MP-SSR be added to the VIVC. We also suggest that the names Perejil tinto (Breval negro (PN)) and Chasselas Rosado de Ye (Chasselas Rosado (PN)) be recorded as new synonyms of either varieties or mutations

of a particular variety. Finally, we recommend that the terms Negra (Alphonse Lavallee (PN)), Negra (Beba roja (PN)), Forastera (Chasselas blanc (PN)), Negra (Listan negro (PN)) and Listan blanca (Palomino fino (PN)) be used as synonyms for the varieties indicated in brackets. These results prove the existence of a large variety of both intervarietal and intra-varietal genetic profiles in the *Vitis vinifera* L. population of Lanzarote. This diversity is the result of the vines brought by the colonisers more than three hundred years ago undergoing a gradual and uninterrupted adaptation process and was favoured by the area not being subject to the phylloxera plague. During this time, natural and human selection, as well as crosses and adaptive mutations, have led to this varietal multiplicity. Furthermore, this evolutionary process has involved the singularisation of the genomes of these individuals, making them increasingly different from their ancestors. Simultaneously, the rich and original Canarian culture has led to the emergence of rich and diverse lexicon associated with this varietal plurality. In short, it can be concluded that the Canary Islands may be one of the few areas in the world where new varieties and clones of *Vitis vinifera* L. have been created. This makes the Canary archipelago, and Lanzarote in particular, an ideal nursery of prepared material to help deal with Climate Change and the high adaptive pressure that the low rainfall and the presence of trade winds exert.

ACKNOWLEDGMENTS

This work was funded by European Agricultural Fund for Rural Development (EAFRD), the Cabildo de Lanzarote and the Consejo Regulador de la Denominación de Origen Protegida Vinos de Lanzarote. The authors are also grateful to Dr. Javier Ibañez, the viticulture specialists Tomás Mesa, Darío Pérez and Ana Garrido, and English language expert Segon Fuentes for their valuable support. We would also like to thank Núria Boronat, Rosa Pastor, Isabel Araque, Braulio Esteve-Zarzoso, Laia Fañanás, and Santiago Moreno for their support in the laboratory.

REFERENCES

Aliquo, G., Torres, R., Lacombe, T., Boursiquot, J.M., Laucou, V., Gualpa, J., Fanzone, M., Sari, S., Pérez-Peña, J., & Prieto, J.A. (2017). Identity and parentage of some South American grapevine cultivars present in Argentina. *Australian Journal of Grape and Wine Research, 23,* 452-460. https://doi.org/10.1111/ajgw.12282

Arroyo-Garcia, R., Ruiz-García, L., Bolling, L., Ocete, R., López, M.A., Arnold C., … (2006). Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. *Mol. Ecol.* 15:3707–3714. https://doi.org/10.1111/j.1365-294X.2006.03049.x

Augusto, D., Ibañez, J., Pinto-Sintra, A.L., Falco, V., Leal, F., Martínez-Zapater, J.M., Oliveira, A.A., & Castro, I. (2021). Grapevine Diversity and Genetic Relationships in Northeast Portugal Old Vineyards. *Plants, 10,* 2755-2779. https://doi.org/10.3390/plants10122755

Bacilieri, R., Lacombe, T., Cunff LL., Di Vecchi-Staraz, M., Laucou, V., Genna, B., Perós, JP., This, P., & Boursiquot, JM. (2013). Genetic structure in cultivated grapevines is linked to geography and human selection. BMC Plant Biol, 13(1), 25. https://doi.org/10.1186/1471-2229-13-25

Balloux, F., & Lugon, N., (2002). The estimation of population differentiation with microsatellite markers. Molecular Ecology 11(2): 155–165. https://doi.org/10.1046/j.0962-1083.2001.01436.x

Bowers, J.E., Dangl, G.S., & Meredith, C.P. (1999b). Development and characterization of additional microsatellite DNA markers for grape. *American Journal of Enology and Viticulture, 50,* 243-246.

Bowers, J.E., Dangl, G.S., Vignani, R., & Meredith, C.P. (1996). Isolation and characterization of new polymorphic simple sequence repeat *loci* in grape (*Vitis vinifera* L.). *Genome, 39,* 628-633. https://doi.org/10.1139/g96-080

Cabezas, A., Ibañez, J., Lijavetzky, D., Vélez, D., Bravo, G., Rodríguez, V., Carreño, I., Jermakow, A.M., Carreño, J., Ruiz-García, L., Thomas, M.R., & Martínez-Zapater, J.M. (2011). A 48 SNP set for grapevine cultivar identification. *BMC Plant Biology,* 11, 153. https://doi.org/10.1186/1471-2229-11-153

Cano, A.A., Yparraguirre, H.C., Del Rocío Torres, M., Navarro, D.S., & Sevillano, R.B. (2021). Molecular and ampelographic characterization of Pisco grapevine accessions conserved in a germplasm collection center of the Ica, Peru | [Caracterización molecular y ampelográfica de accesiones de vides pisqueras conservadas en un centro de colección de germoplasma de Ica, Perú]. *Scientia Agropecuaria*, 12, 525-533. https://doi.org/10.17268/sci.agropecu.2021.057

Crespan, M. (2003b). The parentage of Muscat of Hamburg. *Vitis,* 42, 193-197. https://doi.org/10.5073/vitis.2003.42.193-197

Cretazzo, E., Moreno Sanz, P., Lorenzi, S., Benítez, M.L., Velasco, L., & Emanuelli, F. (2022) Genetic Characterization by SSR Markers of a Comprehensive Wine Grape Collection Conserved at Rancho de la Merced (Andalusia, Spain). *Plants*, 11, 1088. https://doi.org/10.3390/plants11081088

Cipriani, G., Spadotto, A., Jurman, I., Di Gaspero, G., Crespan, M., Meneghetti, S., Frare, E., Vignani, R., Cresti, M., Morgante, M., Pezzotti, M., Pe, E., Policriti, A., & Testolin, R. (2010). The SSR-based molecular profile of 1005 grapevine (*Vitis vinifera* L.) accessions uncovers new synonymy and parentages and reveals a large admixture amongst varieties of different geographic origins. *Theoretical and Applied Genetics*, 121, 1569-1585. https://doi.org/10.1007/s00122-010-1411-9

Dalbó, M.A., Ye, G.N., Weeden, N.F., Steinkellner, H., Sefc, K.M., & Reisch, B.I. (2000). A gene controlling sex in grapevines is placed on a molecular marker-based genetic map. *Genome*, 43, 333-340.

De Oliveira, G.L., De Souza, A.P., De Oliveira, F.A., (...), De Souza, L.M., & Moura, M.F. (2020). Genetic structure and molecular diversity of Brazilian grapevine germplasm: Management and use in breeding programs. Open Access. *PLoS ONE*, 15, e0240665. https://doi.org/10.1371/journal.pone.0240665

Emanuelli, F., Lorenzi, S., Grzeskowiak, L., Catalano, V., Stefanini, M., Troggio, M., Myles, S., Martinez-Zapater, JM., Zyprian, E., Moreira, FM., & Grando, S. (2013). Genetic diversity and population structure assessed by SSR and SNP markers in a large germplasm collection of grape. BMC Plant Biol, 13, 39. https://doi.org/10.1186/1471-2229-13-39

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology,* 14, 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Falush, D., Stephens, M., & Pritchard, J.K. (2003). Inference of population structure using multilocus genotype data: linked

loci and correlated allele frequencies. *Genetics*, 164, 1567-1587. https://doi.org/10.1093/genetics/164.4.1567

Forni, G. (2012). The origin of ''Old World'' viticulture. (Maghradze D, Rustioni L, Turok J, Scienza A, Failla O (eds) In The Caucasus and northern Black Sea region ampelography. *Vitis*, *Special Issue, JKI* - Julius Kuhn-Institut). https://doi.org/10.5073/vitis.2012.51.special-issue.3-481

Fort, F., Hayoun, L., Valls, J., Canals, J.M., Arola, L., & Zamora, F. (2008). A new and simple method for rapid extraction and isolation of high-quality RNA from grape (*Vitis vinifera*) berries. *Journal of the Science of Food and Agriculture*, 88, 179-184. https://doi.org/10.1002/jsfa.3066

Gladstones, J. (2011). Wine, Terroir and Climate Change. Wakefield Press, Kent Town, South Australia.

Ibañez, J., De Andrés, M.T., Molino, A., & Borrego, J. (2003). Genetic study of key Spanish grapevine varieties using microsatellite analysis. *American Journal of Enology and Viticulture*, 54, 22-30.

IPCC (2014). Climate Change 2014. In: Impacts, Adaptation, and Vulnerability. Eds Field, C. B. *et al*.: Cambridge Univ. Press, Cambridge, and New York.

Jimenez, C., Peiro, R., Yuste, A., García, J., Martínez-Gil, F., & Gisbert, C. (2019). Looking for old grapevine varieties. *Vitis,* 58, 59–60. https://doi.org/10.5073/vitis.2019.58.59-60

Jimenez-Cantizano, A., Garcia de Lujan, A., & Arroyo-Garcia, R. (2018). Molecular characterization of table grape varieties preserved in the Rancho de la Merced Grapevine Germplasm Bank (Spain). *Vitis,* 57, 93–101. https://doi.org/10.5073/vitis.2018.57.93-101

Jimenez-Cantizano, A., Muñoz-Martín, A., Amores-Arrocha, A., Sancho-Galán, P., & Palacios, V. (2020). Identification of red grapevine cultivars (*Vitis vinifera* L.) preserved in ancient vineyards in Axarquia (Andalusia, Spain). *Plants,* 9, 1572,1-10. https://doi.org/10.3390/plants9111572

Jones, G.V. (2003). Climate and terroir: Impacts of climate variability and change on wine. Geoscience Canada: Paper 4 from the "Terroir, Geology and Wine: A Tribute". Session Held at The Geological Society of American Annual. Meeting, Seattle, Washington.

Lefort, F., Kyvelos, C., Zervou, M., Edwards, K., & Roubelakis-Angelakis, K. (2002). Characterization of new microsatellite *loci* from *Vitis vinifera* and their conservation in some *Vitis* species and hybrids. *Molecular Ecology Resources,* 2, 20-21.

Lipper, L., Thornton, P., Campbell, B. M., Baedeker, T., Braimoh, A., Bwalya, M., … (2014). Climate-smart agriculture for food security. *Nature Climate Change,* 4, 1068–1072.

Lobell, D. B., & Schlenker, W., Costa-Roberts, J. (2011). Climate trends and global crop production since 1980. *Science,* 333, 616–620. https://doi.org/10.1126/science.1204531

Lopes, M.S., Rodrigues dos Santos, M., Eiras Dias, J.E., Mendonça, D., & da Câmara Machadoa, A. (2006). Discrimination of Portuguese grapevines based on microsatellite markers. *Journal of Biotechnology*, 127, 34-44. https://doi.org/10.1016/j.jbiotec.2006.06.006

Macias, A. (2005). El paisaje vitícola de Canarias. Cinco siglos de historia. *Ería*, 68, 351-364. https://doi.org/10.17811/er.0.2005.351-364

Marsal, G., Baiges, I., Canals, J.M., Zamora, F., & Fort, F. (2011). A fast, efficient method for extracting DNA from leaves, stems, and seeds of *Vitis vinifera* L. *American Journal of Enology and Viticulture*, 62, 376-381. https://doi.org/10.5344/ajev.2011.10082

Marsal, G., Boronat, N., Canals, J.M., Zamora, F., & Fort, F. (2013). Comparison of the efficiency of some of the most usual DNA extraction methods for woody plants in different tissues of *Vitis vinifera* L. *J Int Sci Vigne Vin*, 47, 227–237. https://doi.org/10.20870/oeno-one.2013.47.4.1559

Marsal, G., Mateo, J.M., Canals, J.M., Zamora, F., & Fort, F. (2016). SSR analysis of 338 accessions planted in Penedes (Spain) reveals 28 unreported molecular profiles of *Vitis vinifera* L. *American Journal of Enology and Viticulture*, 67, 466-470. https://doi.org/10.5344/ajev.2016.16013

Marsal, G., Bota., J, Martorell., A, Canals, J.M., Zamora, F., & Fort, F. (2017). Local cultivars of *Vitis vinifera* L. in Spanish Islands: Balearic Archipelago. *Scientia Horticulturae* 226, 122-132. https://doi.org/10.1016/j.scienta.2017.08.021

Marsal, G., Mendez, J.J., Mateo-Sanz, J.M., Ferrer, S., Canals, J.M., Zamora, F., & Fort F. (2019). Molecular characterization of *Vitis vinifera* L. local cultivars from volcanic areas (the Canary Islands and Madeira) using SSR markers. *Oeno One*, 4, 667-680. https://doi.org/10.20870/oeno-one.2019.53.4.2404

Maul, E., & Röckel, F. (2015). *Vitis* International Variety Catalogue. http://www.vivc.de [accessed 15/04/2022].

Meirmans, P.G. (2012). Amova-Based Clustering of Population Genetic Data. *Journal of Heredity*, 103(5), 744-50. https://doi.org/10.1093/jhered/ess047

Milla-Tapia, A., Gómez, S., Moncada, X., León, P., Ibacache, A., Rosas, M., Carrasco, B., Hinrichsen, P., & Zurita-Silva, A. (2013). Naturalized grapevines collected from arid regions in Northern Chile exhibit a high level of genetic diversity. *Australian Journal of Grape and Wine Research*, 19, 299–310. https://doi.org/10.1111/ajgw.12020

Moita, A., Santos, R., & Catarina, A. (2018). Unraveling the origin of *Vitis vinifera* L. Verdelho. *Australian Journal of Grape and Wine Research*, 24, 450-460. https://doi.org/10.1111/ajgw.12353

Myles, S., Boyko, A.R., Owens, C.L., Brown, P.J., Grassi, F., Aradhya, M.K., Prins, B., Reynolds, A., Chia, J-M., Ware, D., Bustamante, C.D., & Buckler E.S. (2011). Genetic structure and domestication history of the grape. *PNAS*, 108, 3457-3458. https://doi.org/10.1073/pnas.1009363108

Nei, M. (1972). Genetic distance between populations. *Am Nat*., 106, 283–291.

Newton, A.C., Akar, T., Baresel, J.P. Bebeli, P.J., Bettencourt, E., Bladenopoulos, K.V., Czembor, J.H., Fasoula, D.A., Katsiotis, A., Koutis, K., Koutsika-Sotirou, M., Kovacs, G., Larsson, H., De Carvalho MAAP, Rubiales, D., Russel, J., Dos Santos T.M.M., & Vaz Patto, M.C. (2010). Cereal landraces for sustainable agriculture. A review. *Agronomy for Sustainable Development,* 30, 237–269. https://doi.org/10.1051/agro/2009032

Ocete, C.A., Arroyo, R., Lovicu, G., Rodríguez-Miranda, A., Valle, J.M., Cantos, M., García, J.L., Lara, M., González de Canales, F., Llompart, J., Martín Rodríguez, E., Weiland, C.M., & Ocete, R. (2019). An inventory of the relic Eurasian wild grapevine populational nuclei in Huelva province (Andalusia, Spain). *Vitis,* 58, 53–57. https://doi.org/10.5073/vitis.2019.58.53-57

Paetkau, D., Calvert, W., Stirling, I., & Strobeck, C. (1995). Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology,* 4, 347-354. https://doi.org/10.1111/j.1365-294x.1995.tb00227.x

Paetkau, D., Slade, R., Burden, M., & Estoup, A. (2004). Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, 13, 55-65. https://doi.org/10.1046/j.1365-294x.2004.02008.x

Peakall, R., & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an

update. *Bioinformatics*, 28, 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Pérez, V., Larrañaga, N., Abdallah, D., Wünsch, A., & Hormaza, JI. (2020). Genetic Diversity of Local Peach (Prunus persica) Accessions from La Palma Island (Canary Islands, Spain). Agronomy, 10, 457. https://doi.org/10.3390/agronomy10040457

Pritchard, J.K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics,* 155, 945-959. https://doi.org/10.1093/genetics/155.2.945

Rodriguez-Torres, I. (2018). Variedades de vid cultivadas en Canarias. Descriptores Morfológicos. Caracterización morfológica, molecular, agronómica y enológica. Ed. Instituto Canario de Investigaciones Agrarias. Gobierno de Canarias. Santa Cruz de Tenerife.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 4, 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sancho-Galán, P., Amores-Arrocha, A., Palacios, V., & Jimenez-Cantizano, A. (2019). Genetical, Morphological, and Physicochemical Characterization of the Autochthonous Cultivar 'Uva Rey' (*Vitis vinifera* L.). *Agronomy*, 9, 563-575. https://doi.org/10.3390/agronomy9090563

Sancho-Galán, P., Amores-Arrocha, A., Palacios, V., & Jimenez-Cantizano, A. (2020). Identification and characterization of white grape varieties autochthonous of a warm climate region (Andalusia, Spain). *Agronomy*, 10, 205. https://doi.org/10.3390/agronomy10020205

Sancho-Galán, P., Amores-Arrocha, A., Jimenez-Cantizano, A., & Palacios, V. (2021). Influence of the presence of grape skins during white wine alcoholic fermentation. *Agronomy*, 11, 452. https://doi.org/10.3390/agronomy11030452

Santamarta, J.C., & Naranjo Borges, J. (2015). Restauración de la Cubierta Vegetal y de Espacios Degradados en la Región de la Macaronesia. In: Singularidades de la región macaronésica. Ed. Colegio de Ingenieros de Montes, Madrid, España.

Scott, K.D., Eggler, P., Seaton, G., Rosseto, M., Abblet, E.M., Lee, L.S., & Henry, R.J. (2000b). Analysis of SSRs derived from grape ESTs. *Theoretical and Applied Genetics,* 100, 723-726.

Sefc, K.M., Regner, F., Turetschek, E., Glössl, J., & Steinkellner, H. (1999). Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome,* 42, 367-373. https://doi.org/10.1139/g98-168

Sehgal, D., Vikram, P., Sansaloni, C.P., Ortiz, C., Saint Pierre, C., & Payne, T. (2015). Exploring and mobilizing the gene bank biodiversity for wheat improvement. *PLoS One*, 10(7), e0132112. https://doi.org/10.1371/journal.pone.0132112

Stajner, N., Tomic, L., Ivanisevic, D., Korae, N., Cvetkovic-Jovanovic, T., Beleski, K., Angelova, E., Maras, V., & Javornik, B. (2014). Microsatellite inferred genetic diversity and structure of Western Balkan grapevines (*Vitis vinifera* L.). *Tree Genetics and Genomes,* 10, 127-140. https://doi.org/10.1007/s11295-013-0670-4

Stanimirovic, B., Djordjević, J.P., Pejin, B., (...), Raičević, P., & Tešić, Ž. (2018). Impact of clonal selection on Cabernet Franc Grape and wine elemental profiles. *Scientia Horticulturae*, 237, 74-80. https://doi.org/10.1016/j.scienta.2018.03.043

This, P., Jung, A., Boccacci, P., Borrego, J., Botta, R., Costantini, L., Crespan, M., Dangl, G.S., Eisenheld, C., Ferreira-Monteiro, F., Grando, S., Ibañez, J., Lacombe, T., Laucou, V., Magalhaes, R., Meredith, C.P., Milani, N., Peterlunger, E., Regner, F., Zulini, L., & Maul, E. (2004). Development of a standard set of microsatellite reference alleles for the identification of grape cultivars. *Theoretical and Applied Genetics,* 109, 1448-1458. https://doi.org/10.1007/s00122-004-1760-3

This, P., Lacombe, T., & Thomas, M.R. (2006). Historical origins and genetic diversity of wine grapes. Trends *Genetics,* 22, 511–519. https://doi.org/10.1016/j.tig.2006.07.008

Thomas, M.R., & Scott, N.S. (1993). Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theoretical and Applied Genetics 86, 985-990. https://doi.org/10.1007/BF00211051

van Leeuwen, C., & Seguin, G. (2006). The concept of terroir in viticulture. *Journal of Wine Research*, 17, 1-10. https://doi.org/10.1080/09571260600633135

Vikram, P., Franco, J., Burgueno-Ferrera, J., Li, H., Sehgal, D., Saint Pierre, C., Ortiz, C., Sneller, C., Tattaris, M., Guzman, C., Sansaloni, C.P., Ellis, M., Fuentes-Davila, G., Reynolds, M., Sonder, K., Singh, P., Payne, T., Wenzl, P., Sharma, A., Bains, N.S., Singh, G.P., Crossa, J., & Singh, S. (2016). Unlocking the genetic diversity of Creole wheat. *Scientific Reports*, 6, 23092. https://doi.org/10.1038/srep23092

Vinos de Lanzarote (2019). La Isla, El Cultivo. DO Lanzarote website. https://dolanzarote.com/ [accessed 22/01/2022].

Vitis Canarias (2015). http://vitiscanarias.com [accessed 22/01/2022]

Wagner, H.W., & Sefc, K.M. (1999). IDENTITY 4.0. Centre for Applied Genetics, University of Agricultural Sciences, Vienna, Austria.

Wolkovich, E.M., García de Cortázar-Atauri, I., Morales-Castilla, I., Nicholas, K.A., & Lacombe, T., (2018). From Pinot to Xinomavro in the world's future wine-growing regions. *Nature Climate Change*, 8, 29-37. https://doi.org/10.1038/s41558-017-0016-6

Yuste, A., Martínez-Gil, F., García-Martínez, S., López Lluch, D., Peiró, R., Jimenez, C., Ruiz, J.J., & Gisbert, C. (2019). Recovering ancient grapevine cultivars in the Spanish provinces of Alicante and Valencia. *Acta Horticulturae,* 1248, 43-46. https://doi.org/10.17660/ActaHortic.2019.1248.6

Zerolo, J., Cabello, F., Espino, A., Borrego, J., Ibañez, J., Rodriguez-Torres, I., Muñoz-Organero, G., Rubio, C., & Hernández, M. (2006). Variedades de Vid de Cultivo Tradicional en Canarias. Ed. Instituto Canario de Calidad Agroalimentaria. Gobierno de Canarias. Santa Cruz de Tenerife, España.