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Genetic Pool of the Cultivated Pear Tree (*Pyrus* spp.) in the Canary Islands (Spain), Studied Using SSR Molecular Markers

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Abstract: The Canary Islands have an enormous richness of crops and varieties, many of them traditional or local, selected for decades by farmers based on the most desirable characteristics. Pear trees were introduced to the Canary Islands presumably in the first years after their Conquest in the 15th century, reaching a high degree of diversification. In this study, to determine the genetic identity of the genus *Pyrus* in the Canary Islands for conservation purposes, 266 pear accessions from the islands of Tenerife, La Palma and Gran Canaria were characterized with 18 SSRs, in addition to 190 genotypes from Galicia, Asturias, wild and commercial varieties as references to detect possible synonyms, genetic relationships and the possible genetic structure. We identified 310 unique genotypes, both diploid and putative triploid, 120 of them present only in the Canary Islands (39%, with 50% clonality). The population structure of the genotypes was analyzed by STRUCTURE 2.3.4 software (Pritchard Lab, Stanford University, Stanford, CA, USA). The dendrogram, by using the Jaccard coefficient and principal component analysis (PCoA), separated the analyzed genotypes into stable groups. One of these groups was formed only by Canarian varieties present at lower altitudes, showing adaptation to low chilling requirements with a significant positive correlation (0.432, $p < 0.01$). This first study of the pear germplasm in the Canary Islands reflects the importance of the group of local cultivars and their need for conservation given they are adapted to their peculiar climatic conditions and have a low number of chill units.

Keywords: autochthonous cultivars; microsatellites; genetic resources; germplasm

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1. Introduction

The pear (*Pyrus* spp.) is one of the most important temperate fruits, with world production in 2020 exceeding 23 million tons. The European Union is the second largest producer region (12%) after China [1]. *Pyrus communis* is the predominant cultivated species in Europe, while in Asia those are *P. pyrifolia*, *P. bretschneideri*, *P. sinkiangensis* and *P. ussurensis* [2]. The world trade is based on a few varieties [3,4]; 'Conference', 'Abate Fetel' and 'Williams' represent more than 65% of European pear production [5]. However, the genetic variability of the genus *Pyrus* is very high [2,6–14], with more than 3000 cultivars maintained in different collections around the world. In Spain, the main pear germplasm banks are located in Galicia at the Mabegondo Agricultural Research Centre (CIAM), at the Agrifood Research and Technology Centre of Aragón (CITA), at the Public University of Navarra (UPNA), at the University of Lleida (UdL) and at the Centre for the Conservation of Agricultural Biodiversity of Tenerife (CCBAT) in the Canary Islands [4,15]. In the last region, most temperate fruit trees were introduced in the first years after the islands'

conquest in the 15th century, with written references to pear tree cultivation since the 16th century [16–18].

The Canary Islands (eight islands and five islets) are located in the Atlantic Ocean, between subtropical coordinates 27–29 north latitude and 13–18 west longitude. Their particularities have favored the development of numerous local agricultural varieties, making the islands one of the richest regions in the world in both agricultural and wild biodiversity. Their latitude, isolation/insular character, wide range of altitudes and volcanic features contribute to the emergence of a wide range of endemisms and agricultural varieties highly adapted to these conditions [19]. In addition, exchanges between continents, together with strong migratory processes, have built up a heritage of agricultural biodiversity of enormous qualitative and quantitative value. However, this agricultural biodiversity is endangered by the progressive abandonment of agriculture, the aging of the rural population and the reduced use of traditional varieties.

To recover the agricultural biodiversity of Tenerife, the CCBAT was created in 2003 by agreement of the Plenary of the Council of Tenerife, within the framework of the Insular Biodiversity Plan 2001–2005. Since its inception, this gene bank has been part of the National Program for the Conservation and Use of Plant Genetic Resources (PCURF) of the National Institute of Agricultural Research and Technologies (INIA). Its main objectives are the conservation and sustainable use of plant genetic resources for food and agriculture, thus avoiding the loss of local agricultural biodiversity by evaluation and documentation of plant genetic resources for use. At present, this bank has more than 3200 accessions of a large number of agricultural genres, with *Pyrus* spp. the most frequent (271). However, Tenerife is not the only island with traditional pear cultivars. The Council of La Palma, through the Agrodiversity Centre (CAP) created in 2005, and the Council of Gran Canaria have also identified and committed to the conservation of their local varieties in the last decade.

The replacement of local varieties with a few modern varieties is one of the main causes of genetic erosion [4], with an associated reduction in genetic variability. Local or traditional varieties have been selected by farmers for decades, who have aimed to adapt them to the specific soil and climatic conditions of each area, to prolong the harvest or for different uses. The study, valorization and conservation of these local varieties is, therefore, essential to avoid the irreplaceable loss of such useful adaptations, along with the knowledge and cultural practices associated with them. A further possible threat is the loss of genes that could be of interest to mitigate the effects of climate change in the future, given many of the varieties are adapted to areas with low chill [20], which are scarce and of interest when considering climatic change [21].

Therefore, this first study on the pear germplasm in the Canary Islands was a priority at the CCBAT, with the main objective a comparative genetic evaluation of the Canarian accessions with conservation purposes, to detect possible synonyms, genetic relationships and the possible genetic structure.

2. Materials and Methods

2.1. Plant Material

In this study, we evaluated 456 accessions (Table S1). Among those were 169 entries from the CCBAT pear collection from the island of Tenerife, 64 accessions from the island of La Palma and 33 from the island of Gran Canaria. We analyzed these to determine the genetic diversity of the genus *Pyrus* in the Canary Islands and detect the most interesting individuals for ex situ conservation, thus preserving the genetic diversity of these local resources. Considering the results obtained in apple (*Malus* spp.) [22], where similarities were found between apples from the Canary Islands and those of northwestern Spain, which bore a common genetic structure, 117 unique genotypes at the CIAM (Galicia) [23], 16 from Asturias and 13 wild genotypes were analyzed as references (Figure 1). In addition, 20 world reference pear varieties used in other previous studies for their importance [7,23,24]—including three varieties of *Pyrus pyrifolia*, one of *P. calleryana* and

another one of *P. salicifolia*—and 24 traditional varieties marketed in Galician nurseries were introduced, too.

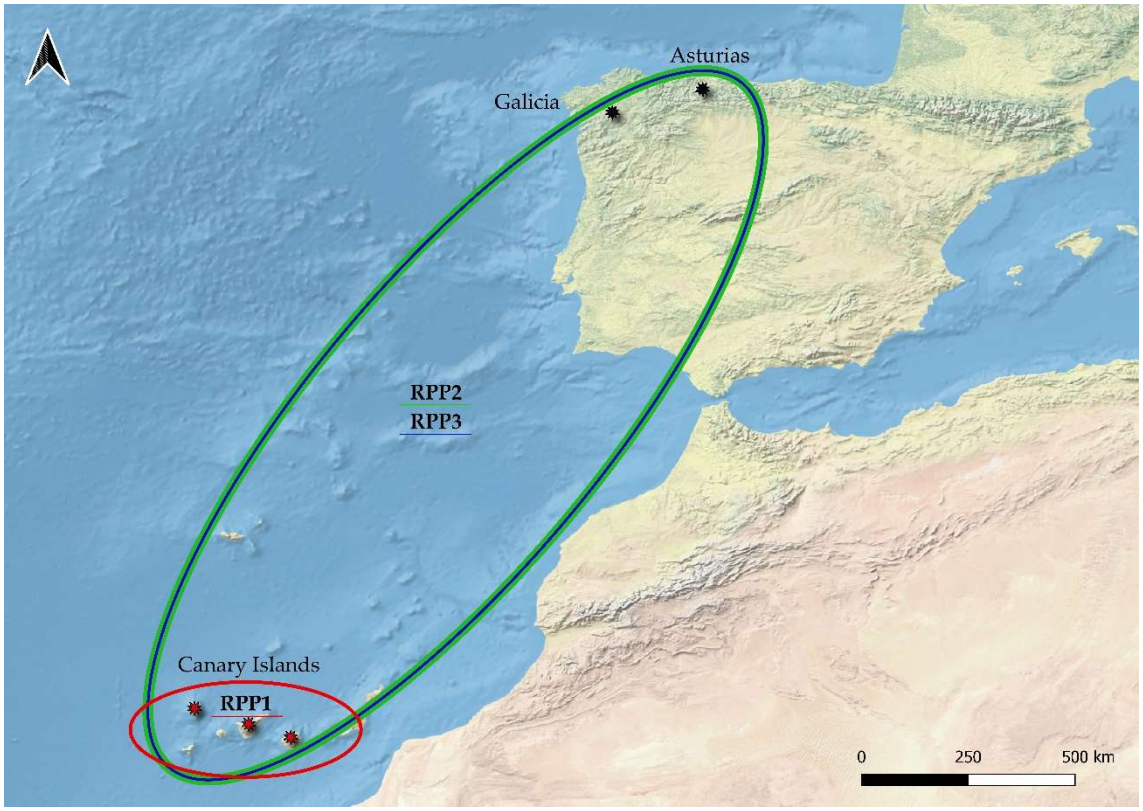

Figure 1. Geographical locations of the 456 samples of pear tree (*Pyrus* spp.) included in this study. RPP1: reconstructed panmictic population unique to the Canary Islands; RPP2, RPP3: reconstructed panmictic populations identified in the northwestern Iberian Peninsula and the Canary Islands.

2.2. DNA Extraction, PCR Reactions, Microsatellite Analysis and Genetic Diversity

DNA extraction was carried out from 50 to 60 mg of young leaves collected in spring/summer and preserved at −80 °C until use. The DNA extraction process was carried out with a buffer based on 2% CTAB, 1% PVP, 100 mM Tris-HCl pH8, 20 mM EDTA pH 8, 1.4 M NaCl and 0.2% beta-mercaptoethanol, with the subsequent addition of chloroform:isoamyl alcohol (CIA) 24:1, which obtained low-quality DNA from most of the samples. Several authors [25,26] referred to the difficulty of extracting quality DNA from different species due to the contents of certain substances such as polyphenols and polysaccharides that act as inhibitors. Therefore, the protocol used was carried out with an extra addition of CIA and subsequent centrifuge to eliminate the inhibitors, thus achieving a higher-quality DNA. However, in 12 samples, the extraction was unsuccessful in this way, meaning it was carried out again with a QIAGEN extraction kit (QIAGEN, Hilden, Germany), with better results. DNA quantification was performed in a Nabi UV/Vis Nanospectrophotometer.

The PCR reaction was performed in 15 µL as the final volume (7.5 µL of QIAGEN Multiplex Master Mix, 0.075 to 0.3 µM of each primer, 4 to 4.9 µL of RNase Free Water and 2 µL of ADN at 10 ng/µL). The samples were amplified in a PTC-100 thermocycler (M.J. Research, Inc.) based on the protocol carried out by Reija [27]. The amplification conditions were 94 °C for 2 min, followed by 35 cycles at 95 °C for 30 s, then the annealing temperature depending on the multiplex set, held for 90 s, followed by 1 min at 74 °C and a final extension at 72 °C for 5 min. The amplification products were diluted with water, and 2 µL of a diluted amplification product was added to 9.88 µL of formamide and 0.12 µL of internal GeneScan^{TM}, size standard 500 LIZ (−250) (Applied Biosystems, Foster City, CA,

USA), then analyzed on a 3130 Genetic Analyzer capillary sequencer (Applied Biosystems, Foster City, CA, USA). The allele sizes were detected using Peak Scanner^{TM} software 1.0 (Applied Biosystems, Foster City, CA, USA).

For the molecular characterization, we used 18 SSRs described by different groups [28–32] (Table S2), previously evaluated by Dos Santos [23] with the exception of CH04c07 (due to the high number of null alleles in that study). Of the 18 SSRs, 14 are recommended by the European Cooperative Programme for Plant Genetic Resources (ECPGR) [33]. For each locus, the total number of alleles, the inbreeding (F_{IS}) and total consanguinity coefficients (F_{IT}), the fixation index (F_{ST}) and the number of migrants (Nm) in GenAlEx 6.2 [34] were analyzed, also identifying possible specific alleles for the Canarian genotypes.

2.3. Assessment of Population Structure

The genetic structure was evaluated by a model-based Bayesian procedure using Structure v2.3.4 software (Pritchard Lab, Stanford University, Stanford, CA, USA) [35], separately for diploid and putative triploid (those with an extra allele) genotypes, following a methodology previously defined and used by several authors [8,36–38]. The analysis was performed for the total of SSRs (18) and also eliminating those that presented null alleles in the work by Dos Santos [23] (CH02b10, CH03d12, CHVF1 and EMPc117) or linked loci (CH01d08 and CH02d11), to confirm the population structure with the two sets of SSRs. In addition to the total number of samples, the genotypes from the Canary Islands were also analyzed separately with the commercial reference varieties alone, to verify the results. We assessed the hypothesis that genotypes could be grouped into between 1 and 14 populations (K). A length of the Markov Monte Carlo Chain (MCMC) race of 1,000,000 steps was used, with 3000 previous steps of dememorization. Each genotype was also considered to have an anonymous origin using the options 'usepopinfo = 0, popflag = 0'. For each K, 30 random iterations were performed. After the analysis, we observed in how many populations the varieties were grouped. Once the most probable K was determined by the methodology described by Evanno et al. [39] using STRUCTURE HARVESTER software [40], the mean ancestry coefficients (*qI*) for each genotype were calculated as an average of the 30 iterations. We considered the minimum value of 0.8 for the allocation to each reconstructed panmictic population (RPP), a value also used by other authors for this and other species [11,12,22–24,41–44]. Those genotypes with $qI < 0.8$ were considered admixed. For each population, the observed (Ho) and expected (He) heterozygosity [45] at each locus were calculated with GenAlEx 6.2 [34] for diploid genotypes. Genodive v2.0b23 software [46] was used to carry out the pairwise reconstructed population differentiation, to obtain Fst values, and an analysis of molecular variance (AMOVA) [47,48], assuming that results based on individuals with two alleles could also be applied to individuals with three alleles [36].

2.4. Genetic Similarity and Principal Component Analysis (PCoA)

The co-dominant SSR data were first converted to a binary data matrix, treating the absence of a defined allele as '0' and presence as '1'. The Jaccard coefficient (JC) was then computed based on the binary data, not considering the shared absence of a character as a similarity [36]. Genotypes were clustered through the unweighted pair group method (UPGMA) [49] and a dendrogram was constructed using the NTSYS 2.21w statistical package (Applied Biostat LLC, Albany, NY, USA) [50]. This was done for 18 and 12 SSRs, and the cophenetic correlation coefficient was subsequently calculated to verify the similarity between the initial matrix and the dendogram, choosing the one made based on the number of microsatellites that obtained a lower distortion.

Principal components (PCs) were estimated on the variance-covariance matrix of the allele frequencies [51,52] using SPSS statistics software v28 (IBM, Armonk, NY, USA).

3. Results

3.1. Microsatellite Analysis and Genetic Diversity

From the total number of samples analyzed in this study (456), 310 unique pear genotypes were identified. The average clonality in this territory was 50%, with island percentages of 47% in Tenerife, 38% in La Palma and 42% in Gran Canaria. Of the samples collected in the Canary Islands, 133 genotypes were found, 120 of them unique to this region (90%). One Canarian genotype was present on the three islands, nine genotypes were located in Tenerife and La Palma, 71 genotypes were exclusive to Tenerife, 28 were from La Palma and 11 were from Gran Canaria, in many cases (70%), with only one accession per genotype. Synonymies were found among the Canarian varieties with the commercial 'Esganacan' (15 accessions), 'Blanquilla' (12), 'Williams' (8), 'Ercolini' (5), 'Portuxesas' (5), 'Precoz de Moretini' (3), 'Manteca temprana' (2), 'Tosca Mediana' (1), 'Roma' (1), 'Rocha' (1), 'Magallón' (1), 'Manteca de oro tardía' (1) and 'San Juan' (1), as well as with a variety ('De manteca') with trees also found in Galicia and Asturias. None of the samples from the Canary Islands were identified as other *Pyrus* species studied.

Both diploid (192) and putative triploid (118) genotypes were detected in the pear samples, but 52% of the putative triploids only presented one locus with three alleles and their ploidy should be confirmed by flow cytometry. The diploid genotypes were distributed as follows: one genotype with trees on the three islands studied, four with trees on Tenerife and La Palma, 35 diploid genotypes unique to the island of Tenerife, 19 from La Palma, nine from Gran Canaria, 73 Galician, 10 Asturian, 10 wild and 31 commercial varieties. Thus, the proportions of diploids and putative triploids in the Canary Islands genotypes were 57 and 43%, respectively.

All the SSR markers studied were polymorphic, with 266 alleles detected in the Canarian accessions, 21 of them not detected in the other samples studied (Table 1). The marker CH05a02 was divided into two loci (a and b) by the existence of two distinct allelic ranges [53], one conceived between alleles 103 and 109 (assigned to CH05a02a) and another between 111 and 131 (assigned to locus CH05a02b).

Alleles specific to the other *Pyrus* species studied were identified. In *Pyrus pyrifolia,* three specific alleles were identified at loci CH01f07a (181), CH03d12 (93) and EMPc11 (143). In 'Pendula' (*Pyrus salicifolia*), seven specific alleles were found: 296 and 305 from locus CH01d08, 112 from CH02d10, 95 from CH03d12, 222 from CH03g07, 117 from locus CH05c06 and 123 from EMPc11. For *Pyrus calleryana* ('Chanticleer'), two specific alleles were detected: 117 from CH04d03 and 171 from EMPc11.

The mean F_{IS} value was −0.05, indicating an excess of heterozygotes, with values between −0.45 for the CH05a02a locus and 0.15 for CH02b10. The lowest total consanguinity coefficient (F_{IT}) was for the ch05a02a locus (−0.42) and the highest for CHVF1 (0.16). The mean F_{ST} (fixation index) per locus was 0.05, with the lowest value (0.02) again for CH05a02a and the highest (0.07) for the loci CH05a02b, CHVF1 and GD142. The number of migrants (Nm) (gene flow) ranged from 3.14 for CH05a02a to 10.00 for CH05c06, with a mean of 5.36 (Table 2).

3.2. Population Structure

3.2.1. Diploids

Among the total number of diploid genotypes studied (192), using the procedure presented by Evanno et al. [39], a higher probability was found for three reconstructed panmictic populations (K = 3) (Figure S1) with both 18 and 12 microsatellites. There was a submaximum for K = 4 in the analysis with 12 SSRs, which may indicate the presence of a substructure, as indicated by various authors in several crops [8,23,39,43,54].

Table 1. Ranges and allelic sizes (pb) for 19 polymorphic loci in the entries studied. The alleles located in the Canarian samples are underlined and those found only in this region are highlighted in bold.

Locus	Allelic Range of Canarian Samples	Allelic Size (pb)	No. of Alleles from Canarian Samples	Total No. of Alleles
CH01d03	130–171	130, 132, 134, 136 [3], 138, 140, **142**, 145, 147, 149 [2], 151 [2], 153, 155, 157, 159, 161, 163, 167, 171, 179, 181, 183, 187, 189, 193 [1], 195, 199, 201	15	28
CH01d08	239–300	239, **248**, **252**, **270**, 276 [1], 278, 279, 280 [2], 282, 284, 286 [3], 288, 290, 292, 294 [3], 296 [1], 300, 305 [1]	13	18
CH01d09	119–161	119, **126**, 128, 130, 132 [1], 134, 136, 138, 140 [2], **142**, 143, 145, 147, 149, 151 [1], 153, 155 [2], 157, 159, 161, 165, 170, 179	19	23
CH01f07a	173–209	171, 173, 175, 177, **179**, 181 [2], 182, 184 [1,] 186, 188, 190, 192 [1], 194 [3], 197, 199 [3], 201, 205, 207 [2], 209, 211, 213, 215, 219	15	23
CH02b10	118–161	112 [1], 116, 118 [3], 120, 122 [2], 124, 126, 128, 130, 132 [2], 134, 136, 138 [1], 141, 143, 145, 147 [3], 149, 151, 153, 155, **159**, 161	17	23
CH02c09	229–283	229, 231, 233 [1], 235, 237, 239, 241, 243, 245 [1], 247 [2], 249 [2], 251 [3], 253 [3], 255 [2], 257, 267, 283	15	17
CH02c11	201–247	201, 205, 207, 209, 211, 215, 217, 219, 221, 223 [13], 225 [2], 227 [12], 229, 231 [3], 233, 235, 237 [2], 239, 241, 243, 245, 247, 249	19	23
CH02d11	95–147	95, 99 [2], 101 [3], 103 [3], 105, 107, 109, 111, 113, 115 [2], 117, 119, 121, 123 [1], 125, 127, 129, 137, **147**, 153	14	20
CH03d12	92–159	92 [3], 93 [2], 95 [1], 97 [2], 101, 103 [1], 106, 108 [3], 110, 112 [2], 114, 116, 118, 120, 122, 125, 127, **129**, 132, 134, 139, **142**, **149**, 157, 159	15	25
CH03g07	204–266	200, 204, 206, 211, 215 [3], 220 [1], 222 [1], 226, 228, **230**, 232, 234, 236, 238, 242, 244, 245, 246, 248, 250 [2], 252 [2], 256, 258 [2], 262, 264, 266, 268	16	27
CH04e03	180–213	177 [3], 180 [1], **186**, 188 [23], 190, 196, 198, 200, 203, 205, **207**, **213**	7	12
CH05a02a	103–109	103, 105 [12], 107 [1], 109	4	4
CH05a02b	111–131	111 [2], 113 [123], 115, 117, 119 [3], 121, 123, 125 [1], 127 [3], 129, 131	10	11
CH05c06	79–114	79, 83 [2], 87, 89, 91, 93, 95, 97 [3], 101, 103 [2], 105 [2], 107, 111, 114 [1], 117, 121	13	16
CH-Vf1	126–172	**126**, 128, 130 [123], 132 [1], 134, 138, 140, 142, 144, 146, 148, 150, 152, 154 [3], 156, 158 [2], 162 [2], **172**	17	18
EMPc11	136–157	123 [1], 130, 134, 136, 138, 140 [2], 142, 143 [2], 144 [23], 146, 149, 151, 153 [2], 155, 157, 171 [3]	10	16
EMPc117	84–139	84, 88, 91 [3], 93 [12], 97, 99, 101 [1], 103, 105 [2], 107 [3], 109, 111, 113, 115, 117, 119, 121, 123, 125, 139	17	20
GD142	126–184	**126**, **134**, 138, 140 [2], 143 [2], 147 [1], 150, 152, 154, 156, 158, 160, 162, 164, 166 [1], 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188 [3], 198, 204	17	28
GD147	125–162	117, 125, 127, 129, 131 [1], 133 [1], 135 [2], 137 [2], 139 [3], 141, 143, 146, **150**, 154, 162	13	15
Total			266	367

[1] alleles detected in *Pyrus salicifolia* genotypes; [2] alleles detected in *P. pyrifolia* genotypes; [3] alleles detected in *P. calleryana* genotypes.

Table 2. Inbreeding coefficient (F_{IS}), total consanguinity coefficient (F_{IT}), fixation index (F_{ST}) and number of migrants (Nm) per locus in 192 diploid pear genotypes.

Locus	F_{IS}	F_{IT}	F_{ST}	Nm
CH01d03	−0.09	−0.05	0.04	6.11
CH01d08	−0.09	−0.05	0.04	7.24
CH01d09	−0.01	0.04	0.05	5.90
CH01f07a	−0.03	0.03	0.06	8.28
CH02b10	0.15	0.20	0.06	5.98
CH02c09	−0.03	0.02	0.05	4.21
CH02c11b	−0.09	−0.04	0.05	4.76
CH02d11	0.09	0.14	0.06	6.51
CH03d12	0.06	0.11	0.05	4.47
CH03g07	−0.04	0.00	0.04	4.13
CH04e03	−0.02	0.01	0.03	4.43
CH05a02a	−0.45	−0.42	0.02	3.14
CH05a02b	−0.37	−0.28	0.07	4.39
CH05c06	−0.01	0.03	0.04	10.00
CHVF1	0.09	0.16	0.07	3.48
EMPc11	−0.14	−0.10	0.03	3.31
EMPc117	0.10	0.15	0.05	4.39
GD142	0.02	0.08	0.07	6.67
GD147	−0.08	−0.04	0.04	4.32
Average	−0.05	0.00	0.05	5.36

For K = 3, one population (RPP1) was formed consisting exclusively of Canarian genotypes (Figure 1). Another population (RPP2) integrated the commercial varieties of *Pyrus communis* 'Mantecosa Hardy', 'Magallón', other varieties marketed in Galician nurseries, the wild diploid genotypes, the two diploid varieties of *Pyrus pyrifolia*, Galician and Asturian genotypes and a few Canarian genotypes. The last population RPP3 was formed by most of the reference commercial varieties, as well as Galician, Asturian and Canarian genotypes. For K = 4 and 12 SSRs, the populations RPP1 and RPP3 remained practically unchanged with only some alterations to the admixed group, while RPP2 was divided into two groups: one with the wild genotypes, Asian pear cultivars, 'Magallón' and some Galician, Asturian and Canarian genotypes (RPP2.1); then, another one (RPP2.2) that included 'Mantecosa Hardy' among other varieties (Table 3) [35].

Velázquez et al. [20] zoned the island of Tenerife based on the dynamic model of Fishman et al. (1987), delineating a coastal zone with practically no winter cold, which begins to increase as the elevation rises. Figure 2 shows the layer of pear trees located on the island and the relation to such zonation, according to the reconstructed panmictic populations for K = 3 and 18 SSRs. RPP1 accessions were collected at lower elevations than the other groups, with a significant positive correlation (0.432, $p < 0.01$). The accessions of that group were collected at an average altitude of 569 m above sea level (m.a.s.l.), RPP2 at 663 m.a.s.l. and RPP3 at an average altitude of 746 m.a.s.l. This shows an apparent adaptation to the low winter chilling requirements of the RPP1 genotypes.

Table 3. Classification of 192 diploid pear genotypes for K = 3 and K = 4 reconstructed populations (RPPs) according to STRUCTURE [35] for the total study sample.

K = 3, 12 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**	**Number of Genotypes of Other Origins**
RPP1 (Canary Islands) qI [1] ≥ 0.8	39 (20.31%)	39	0	0
RPP2 ('Mantecosa Hardy') qI [1] ≥ 0.8	71 (36.98%)	5	11	55
RPP3 (Commercial) qI [1] ≥ 0.8	51 (26.56%)	10	15	26
Admixed (qI [1] < 0.8)	31 (16.15%)	14	5	12
Total	**192 (100.00%)**	**68**	**31**	**93**
K = 3, 18 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**	**Number of Genotypes of Other Origins**
RPP1 (Canary Islands) qI [1] ≥ 0.8	39 (20.31%)	39	0	0
RPP2 ('Mantecosa Hardy') qI [1] ≥ 0.8	67 (34.90%)	9	12	46
RPP3 (Commercial) qI [1] ≥ 0.8	53 (27.60%)	12	15	26
Admixed (qI [1] < 0.8)	33 (17.19%)	8	4	21
Total	**192 (100.00%)**	**68**	**31**	**93**
K = 4, 12 SRRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**	**Number of Genotypes of Other Origins**
RPP1 (Canary Islands) qI [1] ≥ 0.8	36 (18.75%)	36	0	0
RPP2.1 (Wild, other *Pyrus*) qI [1] ≥ 0.8	31 (16.15%)	5	3	23
RPP2.2 ('Mantecosa Hardy') qI [1] ≥ 0.8	35 (18.23%)	1	5	29
RPP3 (Commercial) qI [1] ≥ 0.8	48 (25.00%)	9	15	24
Admixed (qI [1] $< 0,8$)	42 (21.87%)	17	8	17
Total	**192 (100.00%)**	**68**	**31**	**93**

[1] Coefficient of ancestry.

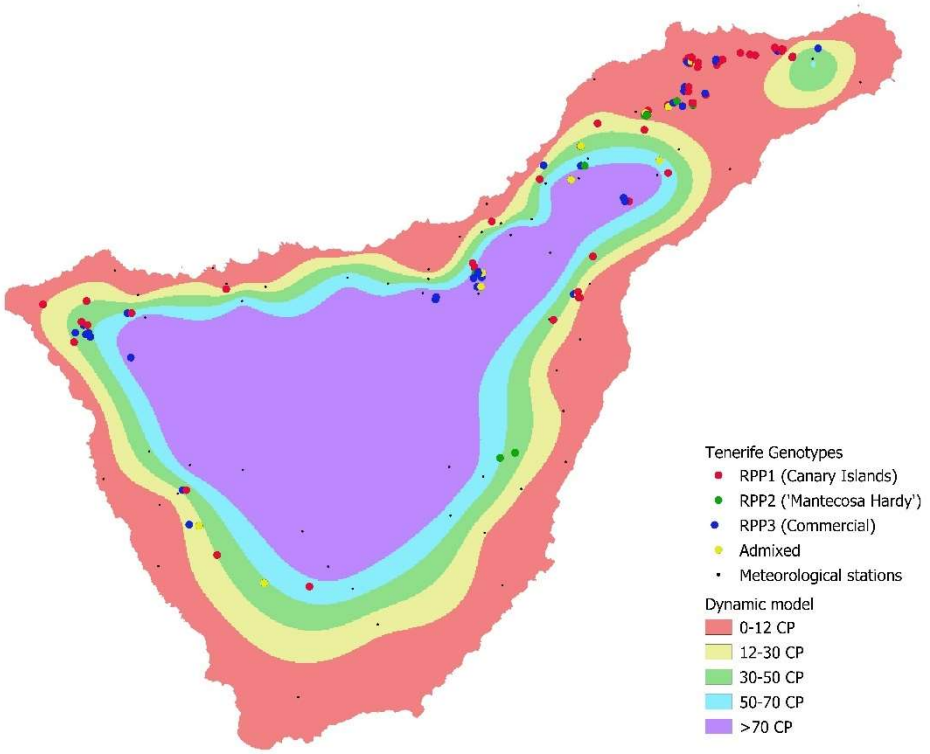

Figure 2. Tenerife genotypes, classified in RPP1-3 and admixed, and their sampling locations in Tenerife, according to the chill portions (CP) of the dynamic model (Fishman et al., 1987) [20].

After reducing the population to the Canarian diploid genotypes and the commercial diploid reference varieties, the maximum probability was for K = 2, with a submaximum in K = 3, and for 18 SSRs, also in K = 6 (Figure S1). In the case of K = 2, the population of Canarian genotypes (RPP1) was separated from the rest, appearing in the admixed group the varieties marketed in Galician nurseries 'Rocha' and 'Tenreiras'. For K = 3, the grouping given for the total number of genotypes was repeated, demonstrating the great stability of these reconstructed panmictic populations (Table 4).

Table 4. Classification of 99 diploid pear genotypes for K = 2, K = 3 and K = 4 reconstructed populations (RPPs) according to STRUCTURE [35] for the Canary Islands genotypes and the reference commercial varieties.

K = 2, 12 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**
RPP1 (Canary Islands) qI [1] ≥ 0.8	39 (39.39%)	39	0
RPP2 (others) qI [1] ≥ 0.8	50 (50.51%)	21	29
Admixed (qI [1] < 0.8)	10 (10.10%)	8	2
Total	**99 (100.00%)**	**68**	**31**
K = 2, 18 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**
RPP1 (Canary Islands) qI [1] ≥ 0.8	39 (39.39%)	39	0
RPP2 (others) qI [1] ≥ 0.8	52 (52.53%)	23	29
Admixed (qI [1] < 0.8)	8 (8.08%)	6	2
Total	**99 (100.00%)**	**68**	**31**
K = 3, 12 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**
RPP1 (Canary Islands) qI [1] ≥ 0.8	38 (38.38%)	38	0
RPP2 ('Mantecosa Hardy') qI [1] ≥ 0.8	21 (21.21%)	10	11
RPP3 (Commercial) qI [1] ≥ 0.8	25 (25.25%)	10	15
Admixed (qI [1] < 0.8)	15 (15.15%)	10	5
Total	**99 (100.00%)**	**68**	**31**
K = 3, 18 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**
RPP1 (Canary Islands) qI [1] ≥ 0.8	39 (39.39%)	39	0
RPP2 ('Mantecosa Hardy') qI [1] ≥ 0.8	23 (23.23%)	11	12
RPP3 (Commercial) qI [1] ≥ 0.8	22 (22.22%)	7	15
Admixed (qI [1] < 0.8)	15 (15.15%)	11	4
Total	**99 (100.00%)**	**68**	**31**
K = 6, 18 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**
RPP1.1 (Canary Islands 1) qI [1] ≥ 0.8	24 (24.24%)	24	0
RPP1.2 (Canary Islands 2) qI [1] ≥ 0.8	12 (12.12%)	12	0
RPP2.1 (Canary Islands 3) qI [1] ≥ 0.8	6 (6.06%)	6	0
RPP2.2 ('Mantecosa Hardy') qI [1] ≥ 0.8	6 (6.06%)	1	5
RPP2.3 (*Pyrus pyrifolia*) qI [1] ≥ 0.8	2 (2.02%)	0	2
RPP3 (Commercial) qI [1] ≥ 0.8	22 (22.22%)	7	15
Admixed (qI [1] < 0.8)	27 (27.27%)	18	9
Total	**99 (100.00%)**	**68**	**31**

[1] Coefficient of ancestry.

For K = 6 and 18 SSRs, the genotypes of RPP1 were divided into two groups, one with 24 and the other with 12 Canarian genotypes with $qI \geq 0.8$. The RPP3 group of K = 3 that included the commercial and related varieties remained practically unchanged, while the second population in that study was divided into three groups: one composed only of Canarian varieties (RPP2.1); another with 'Mantecosa Hardy', some varieties marketed in Galician nurseries and a Gran Canarian genotype (RPP2.2); a third group formed by *Pyrus pyrifolia* (RPP2.3) (Table 4).

3.2.2. Triploids

For the putative triploids (118) and 12 SSRs, the highest probability was for K = 2, with a submaximum at K = 3, while for the total number of microsatellites, there was only a maximum at K = 3 (Figure S1). For K = 2 (12 SSRs), practically (6.06%) the same types of groups were formed as those for the diploids, separating a group of Canarian varieties (RPP1) from the rest, although in this case, a Galician variety ('Rabuda parda') was introduced in RPP1 with $qI \geq 0.8$. For K = 3 and 12 SSRs, the RPP2 of K = 2 was divided into two groups, one of them with the wild triploid genotypes, the triploid varieties of *Pyrus pyrifolia*, *P. salicifolia* and *P. calleryana*, 'Castell', two marketed in the Galician nurseries 'Barburiña xermade' and 'Urraca amarela', as well as Galician, Asturian and four Canary Island varieties. The other group (RPP3) was formed by Canarian, Asturian and Galician genotypes, some nursery varieties and the reference variety 'Roma'. For K = 3 and 18 SSRs, 'Rabuda parda' presented a *qI* lower than 0.8, so RPP1 was formed only of Canarian genotypes, and the other two groups were similar to those obtained with 12 microsatellites (Table 5).

Table 5. Classification of 118 triploid pear genotypes for K = 2 and K = 3 reconstructed populations (RPPs) according to STRUCTURE [35] for the total study sample.

K = 2, 12 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**	**Number of Genotypes of Other Origins**
RPP1 (Canary Islands) qI [1] ≥ 0.8	34 (28.81%)	33	0	1
RPP2 (others) qI [1] ≥ 0.8	77 (65.25%)	17	14	46
Admixed (qI [1] < 0.8)	7 (5.93%)	2	0	5
Total	**118 (100.00%)**	**52**	**14**	**52**
K = 3, 12 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**	**Number of Genotypes of Other Origins**
RPP1 (Canary Islands) qI [1] ≥ 0.8	33 (27.97%)	32	0	1
RPP2 ('Castel', other *Pyrus*) qI [1] ≥ 0.8	29 (24.58%)	4	6	19
RPP3 (Local varieties) qI [1] ≥ 0.8	40 (33.90%)	12	8	20
Admixed (qI [1] < 0.8)	16 (13.56%)	4	0	12
Total	**118 (100.00%)**	**52**	**14**	**52**
K = 3, 18 SSRs	**Total Number of Genotypes (% in Brackets)**	**Number of Canarian Genotypes**	**Number of Reference Genotypes**	**Number of Genotypes of Other Origins**
RPP1 (Canary Islands) qI [1] ≥ 0.8	31 (26.27%)	31	0	0
RPP2 ('Castel', other *Pyrus*) qI [1] ≥ 0.8	33 (27.97%)	6	6	21
RPP3 (Local varieties) qI [1] ≥ 0.8	32 (27.12%)	9	5	18
Admixed (qI [1] < 0.8)	22 (18.64%)	6	3	13
Total	**118 (100.00%)**	**52**	**14**	**52**

[1] Coefficient of ancestry.

When studying only the triploids of the Canary Islands and the reference triploid varieties (66 genotypes), two populations (K = 2) (Figure S1) were separated for 12 and 18 SSRs: RPP1 of Canarian genotypes and the rest.

From the Canary Islands group (RPP1), the varieties with the highest number of specimens were 'Güimarera or Sanjuanera' (9), 'Parda' (8) and 'Rabuda or Calabazate' (8), all with trees on Tenerife and La Palma.

3.2.3. Genetic Diversity in Reconstructed Panmictic Populations (RPPs)

The mean values of observed (Ho) and expected (He) heterozygosity were similar for RPPs and the admixed genotype group (Table 6). The lowest He was for the locus CH04e03 in RPP2 (0.33), although the lowest value was recorded in the admixed group, with 0.30. The maximum He value was also recorded in RPP2, in the loci CH03g07 and GD142. The total range of He was from 0.38 to 0.86 with an average value of 0.76. The mean Ho was higher than He in RPP1 and RPP3, as well as in the total average. RPP1 had the lowest He average (0.70) and the highest Ho value (0.84).

Table 6. Observed (Ho) and expected (He) heterozygosity in reconstructed panmictic populations (RPPs) of 192 diploid pear genotypes for K = 3 and 18 SSRs.

	RPP1		**RPP2**		**RPP3**		**Admixed**		**Total**	
	Ho	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**	**Ho**	**He**
CH01d03	0.97	0.80	0.85	0.90	0.94	0.82	0.91	0.85	0.92	0.84
CH01d08	0.86	0.69	0.76	0.82	0.92	0.80	0.81	0.76	0.84	0.77
CH01d09	0.89	0.80	0.78	0.90	0.94	0.85	0.88	0.91	0.87	0.86
CH01f07a	0.87	0.65	0.83	0.90	0.80	0.80	0.84	0.89	0.84	0.81
CH02b10	0.78	0.76	0.71	0.87	0.67	0.83	0.67	0.87	0.71	0.83
CH02c09	0.97	0.75	0.85	0.88	0.63	0.62	0.70	0.78	0.79	0.76
CH02c11b	0.94	0.81	0.88	0.91	0.94	0.81	0.97	0.88	0.93	0.85
CH02d11	0.58	0.65	0.61	0.82	0.84	0.79	0.79	0.84	0.70	0.78
CH03d12	0.92	0.78	0.63	0.88	0.71	0.68	0.73	0.84	0.74	0.79
CH03g07	0.90	0.83	0.87	0.92	0.96	0.83	0.83	0.86	0.89	0.86
CH04e03	0.53	0.44	0.24	0.33	0.46	0.44	0.31	0.30	0.38	0.38
CH05a02a	1.00	0.66	1.00	0.73	1.00	0.72	1.00	0.65	1.00	0.69
CH05a02b	1.00	0.62	1.00	0.78	1.00	0.76	1.00	0.77	1.00	0.73
CH05c06	0.67	0.57	0.71	0.79	0.76	0.74	0.70	0.71	0.71	0.70
CHVF1	0.63	0.66	0.73	0.83	0.75	0.79	0.73	0.85	0.71	0.78
EMPc11	0.87	0.71	0.88	0.82	0.81	0.71	0.85	0.76	0.85	0.75
EMPc117	0.89	0.82	0.57	0.90	0.84	0.80	0.70	0.83	0.75	0.84
GD142	0.84	0.75	0.88	0.92	0.79	0.82	0.79	0.87	0.83	0.84
GD147	0.79	0.62	0.76	0.77	0.47	0.46	0.76	0.72	0.70	0.64
Average	0.84	0.70	0.76	0.82	0.80	0.74	0.79	0.79	0.80	0.76

The AMOVA analysis showed that the allelic variability between populations was 10.8% ($p < 0.001$). The largest difference between populations (Fst) was presented by RPP1 and RPP3, with a value of 0.099, followed by differences between RPP1 and RPP2 of 0.068 and RPP2 and RPP3 of 0.052, with significant differences in all cases (Table 7).

Table 7. Differentiation between pairs of populations (Fst) in diploids for K = 3, 18 SSRs.

RPP1 (Canary Islands)	RPP2 ('Mantecosa Hardy')	RPP3 (Commercial)	Admixed	
0.068 ***	-			**RPP2 ('Mantecosa Hardy')**
0.099 ***	0.052 ***	-		**RPP3 (Commercial)**
0.051 ***	0.011 ***	0.026 ***	-	**Admixed**

*** p <0.001.

The reconstructed panmictic population RPP2, which included different species of the genus *Pyrus*, had the highest number of specific alleles (94). In RPP1 genotypes, three alleles not found in other populations were detected: 129 of the CH03d12 locus (five genotypes), 230 of CH03g07 (1) and 126 of CHVF1 (1). The most frequent unique Canarian allele was 142 of CH03d12, identified in one cultivar from Tenerife and La Palma, seven genotypes from Tenerife and five from La Palma, all from RPP1 except one admixed cultivar.

3.3. Genetic Similarity and Principal Component Analysis (PCoA)

The cophenetic correlation coefficient was higher for the study with the total number of microsatellites (0.77) than with the small set of 12 SSRs (0.73), so the dendogram made from the Jaccard coefficient with 18 SSRs was chosen (Figure 3). These coefficient values reflected some degree of distortion between the initial matrix and the representation, but nevertheless, the results were in accordance with those obtained in the population structure. The dendogram obtained grouped all the genotypes of RPP1, both diploid and triploid, in a single cluster from a Jaccard coefficient (JC) of 0.21. In that cluster, in addition to these genotypes, only two genotypes of the admixed group from Tenerife and the varieties marketed in Galician nurseries 'Rocha' and 'Tenreiras' (two clones) were included, which were again not assigned to any reconstructed panmictic population. This cluster was grouped, in turn, from 0.19 with three Galician varieties (CIAM OU231, CIAM LU214 and 'Peros Raposos') and from 0.18 with two large groups: one mainly comprising the reference and related commercial varieties that formed RPP3 in the results obtained with the STRUCTURE software, and another formed by varieties belonging to RPP2, including 'Mantecosa Hardy'. In the dendogram, RPP2 is divided into two clusters: the one mentioned above and another one containing the most differentiated genotypes and other *Pyrus* species. The groupings provided by STRUCTURE for K = 6 and 18 SSRs in diploids, although obtained by studying the Canarian genotypes and reference commercial varieties and not the total number of the samples, can also be differentiated in the dendogram: in its upper part are the genotypes of RPP2.2 that integrates 'Mantecosa Hardy' and others, while at the bottom are the other two subgroups (RPP2.3 of *Pyrus pyrifolia*, and RPP2.1 formed by Canarian genotypes). In addition, one of the groups that form the cluster of the genotypes of RPP1, from JC = 0.28, is formed only by members of RPP1.1, so the presence of this substructure seems to be corroborated. The most differentiated genotypes were from Gran Canaria (GC81 'Del País', GC1037 'Pero' and GC17 'Antiguo'), which had seven, five and four unique alleles, respectively, grouped in a cluster with 'Chanticleer' (*Pyrus calleryana*) from JC = 0.09. These may correspond to genotypes of *Pyrus* species different from those recognized and introduced as references in this study, or they may be hybrids of them.

The representation of the principal components (PCs) also separated the reconstructed panmictic populations quite clearly, with the admixed genotypes between the RPPs (Figure 4). RPP1 showed negative values for Factor 1, with only specimens of this group (70% of its members) appearing below F1 = −1.3, while 100% of the genotypes grouped in RPP3 obtained by STRUCTURE for K = 3 and 18 SSRs (diploid and putative triploid) had an F1 value greater than 0.11. The allele that mostly contributed to Factor 1 was 119 at locus CH05a02b (−0.086), present in *Pyrus calleryana*, wild genotypes and some Galician and Asturian varieties including 'Rabuda parda' and 'Tenreiras'. This allele was very frequent in RPP1 (76%), less frequent in RPP2 (14%) and rare (<5%) in RPP3.

Figure 3. Dendogram for the 310 unique pear genotypes based on Jaccard's coefficient with an indication of the reconstructed panmictic populations for K = 3 and 18 SSRs. The RPP number assigned by the STRUCTURE software is indicated before the genotype code/name.

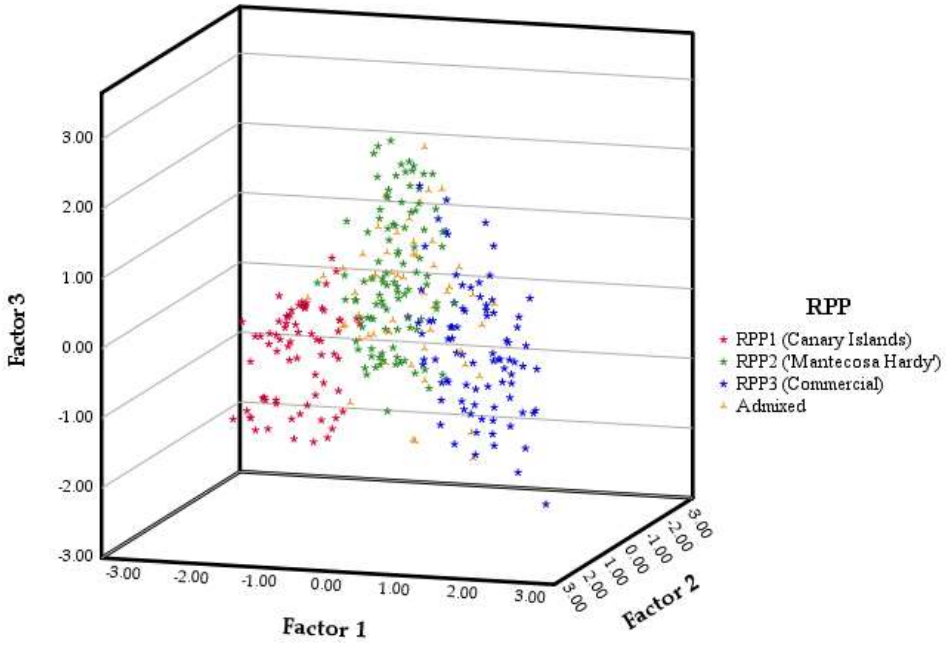

Figure 4. Representation of principal components (PCs) of reconstructed panmictic populations (RPP1 to RPP3) obtained with STRUCTURE using 18 SSRs and 310 pear genotypes.

Genotypes with JC $\leq$ 0.13 (mostly from the RPP2 group) were the most differentiated by the PCoA in the positive Factor 2 (Figure 5) due to specific alleles that grouped genotypes. The allele with the highest coefficient (0.89) was 130 of the CHVF1 locus, present in *Pyrus pyrifolia*, *P. calleryana*, *P. salicifolia*, wild genotypes, seven Galician nursery varieties, five Asturian, many Galician and 26 genotypes from the Canary Islands (six from RPP1, 10 from RPP2, seven from RPP3 and three admixed); on the other hand, the negative coefficient was 245 for CH02c09 ($-$0.134), not present in *P. pyrifolia* or *P. calleryana*.

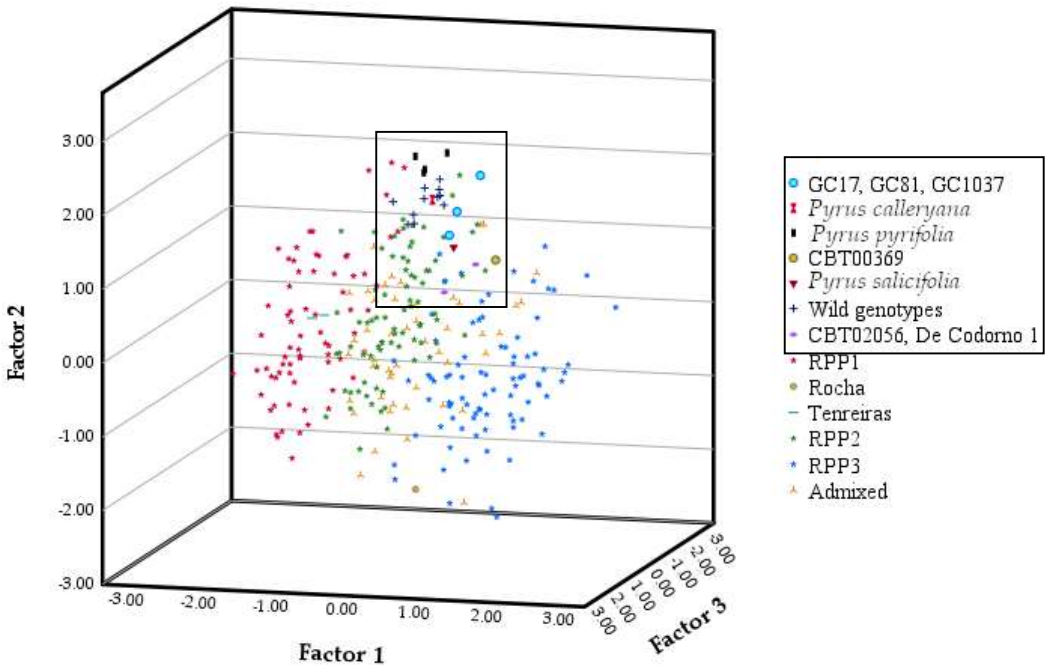

Figure 5. Three first principal components (PCs) of the PCoA for the genotypes differentiated with JC $\leq$ 0.13 and the RPPs using STRUCTURE for 310 pear genotypes.

4. Discussion

4.1. Clonality and Putative Triploids in the Canary Islands' Genotypes

The average clonality in the Canary Islands was 50%, with the percentage for each island varying (38 to 47%) due to the existence of genotypes with individuals on several islands and synonymies found in them. This value was slightly higher than that obtained by Dos Santos [23] (43%) at the CIAM pear germplasm bank and much higher than those of the Public University of Navarra (UPNA) [11] or the Agrifood Research and Technology Centre of Aragón (CITA) [7], both with values below 15%. However, it is similar to the clonality obtained in Spanish apple genebanks [38,55]. Despite the fact that it can be considered high clonality, this parameter has been higher in other studies on *Prunus persica* on the island of La Palma [55], *Castanea sativa* in Spain [56] and *Juglans regia* in China [57], all with clonality higher than 65% (and higher than 85% in the last case). This type of study is highly important for germplasm banks to optimize their management and conservation of the material they hold.

The proportion of putative triploids in the Canarian genotypes (43%) was similar to that obtained by Ferradini et al. [58] (45%) and slightly higher than that of Dos Santos [23] (38% in Galician cultivars). Considering only those with three alleles at more than one locus (25), the percentage of hypothetical triploids decreased to 21%, similar to Ferradini et al. [58] (20%) and Dos Santos [23] (18%) when considering only these types of genotypes, and lower than that found by Bielsa et al. [12] (33%) and higher than Queiroz et al. [59] (8%).

4.2. Bayesian Method Identified a Canarian Cluster of Pear Genotypes

The results of the population structure analysis were in agreement with the data obtained by Dos Santos [23], except for the new RPP1 group formed exclusively by Canarian genotypes. This structure was corroborated by the dendogram obtained from the Jaccard coefficient, grouping the diploid and putative triploid genotypes of this population in the same cluster from JC = 0.21, in which only two varieties marketed in Galician nurseries of Portuguese origin ('Rocha' and 'Tenreiras') were included. In this representation, the genotypes of RPP3 were also grouped, and a division of RPP2, also obtained in some of the studies carried out to determine the population structure, was observed. Principal component analysis (PCoA) then separated the reconstructed panmictic populations, identifying the most determinant alleles for each component.

The accessions of the Canarian group (RPP1) were located at a lower average altitude and chill than the other two reconstructed panmictic populations, indicating a potentially better adaptation of these to warmer areas, maybe as a result of their selection and conservation by farmers over a long period due to these characteristics [4,19]. In a context of potential climate change, the good adaptation of temperate fruit varieties to lower chilling requirements is very important in their areas of origin. This has been one of the objectives of genetic breeding programs in recent decades, which have used wild species relatives or local varieties as parents to increase the available genetic pool on crosses [4,21,60]. The origin of the Canary Islands population is unknown, and it would be interesting in the future to analyze it with samples from other parts of Spain and countries such as Portugal since it may be related to varieties from these origins, and it may include other species of the genus present in the Iberian Peninsula, such as *Pyrus bourgaeana* or *P. cordata*.

4.3. Uniqueness of the Canary Islands' Genotypes

Ninety percent of the genotypes found in the Canary Islands were unique to this territory, most of them (70%) with only one accession, which reflects the high vulnerability of this material. The pear variety with the highest number of entries (15) was 'Esganacan', a variety of Galician origin that appears in the list of varieties with an officially recognized description of a pear tree by the Ministry of Agriculture, Fisheries and Food (MAPA) [61], indicating that its synonym is 'Manteca Oscura'. Fourteen accessions of this genotype were collected on the island of Tenerife, with the names 'De agua' (7), 'Trigal' (2), 'De palo' (2), 'Calabazate', 'Chasnera' or, simply, 'peral', and another accession was collected on Gran

Canaria ('De vino'). This genotype was also the one with the highest number of accessions (14) in the study carried out by Dos Santos [23] in the CIAM collection, which indicates the expansion of this variety and the close historical relationship between Galicia and the Canary Islands. A similar value was found for 'Blanquilla', a widely produced variety of Spanish origin. Both genotypes are part of the RPP3 group (Commercial). From the Canary Islands group (RPP1), the varieties with the highest number of accessions were 'Güimarera or Sanjuanera', 'Parda' and 'Rabuda or Calabazate', all with trees in Tenerife and La Palma and recorded by Viera y Clavijo in the 18th century [62]. In the first references to the cultivation of pear trees in the Canary Islands, in the 15th century, some 'brown pears' ('peras pardas') were mentioned on these two islands [16,17]. The commercial variety Williams (RPP3) was also widely found (eight accessions in Tenerife and Gran Canaria). This last variety is often called 'Buen Cristiano', and there are records of a variety with this name in Tenerife in the 18th century [63]. In 1769, the butler of the Farm Las Palmas de Anaga sent his owner six-dozen 'Buen Christiano' (sic) and 'Pardas' pears, and in 1977, scions were taken in Taganana (Tenerife) of 'Españolas', 'Buen Christiano' (sic) and 'Pardas' pear trees. The variety 'Española' was also analyzed in this study and included in RPP1.

A total of 266 alleles were detected in the Canarian accessions, with 21 not detected in the rest of the samples. Of the seven alleles cited by Dos Santos [23] as unique to *Pyrus pyrifolia*, four were found to be shared with other genotypes in this study: allele 255 of CH02c09 was also identified in two wild genotypes; allele 83 of CH05c06 in nine genotypes from Tenerife and La Palma, eight of them belonging to RPP1 and the remaining one to RPP2; alleles 158 and 162 of the CHVF1 locus were located in one genotype from Tenerife and another from Gran Canaria (GC1037), respectively, both from RPP2. Alleles 140 and 143 of GD142 were also located in the Canary Islands in one genotype from Gran Canaria and another from Tenerife, both of RPP2, which were shared by the Galician variety 'Portuguesa'. In *P. salicifolia*, Dos Santos [23] located 10 specific alleles, and in this study, we found three of them in Canarian genotypes: allele 233 of CH02c09 was identified in one genotype from Tenerife and one from Gran Canaria, both belonging to RPP1; 123 of CH02d13 was located in a wild specimen and one genotype from Tenerife, along with that reconstructed panmictic population; 114 of the CH05c06 locus was also found in a genotype from Gran Canaria of RPP2, being one of the most differentiated in the dendogram from the Jaccard coefficient (GC1037). In *P. calleryana* ('Chanticleer'), two specific alleles were detected out of the five located by Dos Santos [23], sharing: allele 253 of CH02c09 with the Canarian genotype GC81, allele 215 of CH03g07 with a Tenerife genotype of RPP1 and the nursery variety 'San Juan', and allele 127 of CH05a02b with a wild genotype and GC81. Only 'Chanticleer' and the genotype GC17 from Gran Canaria had allele 231 at locus CH02c11.

The mean heterozygosity values were similar to others previously obtained in pear germplasm studies [6,11,23,64]. The mean Ho was higher than He, a fact also observed in studies carried out in Portugal and Sardinia [9,59,65,66], but not in Spain [7,11,23] where He was higher than Ho. This excess of heterozygotes, contributed to by the inbreeding coefficient, indicates high genetic variability.

5. Conclusions

This first study of the pear germplasm in the Canary Islands indicated that the main species cultivated is, as expected, *Pyrus communis*. Yet, SSRs evaluated from a broad range of samples enabled us to identify specific alleles for Asian species, which might be helpful for managing the germplasm in Spain and abroad.

Molecular markers led us to identify a reconstructed panmictic population formed only by Canarian genotypes, including some diploid and others putative triploid, which could be historically traced to the 16th century in written texts. These indicate its presumed introduction from the Iberian Peninsula, which must be checked to determine its origin. This group will be considered a key group of the Canarian genotypes to be conserved at the germplasm bank. Moreover, the correlation found with lower chilling units corroborates

the uniqueness of this Canarian cluster and its relevance for warmer areas, or those at risk of effects of climate change, and for breeding programs needing a broader genetic pool for crosses.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12071711/s1, Figure S1: ΔK values, defined by Evanno et al.'s method [39] and obtained using STRUCTURE HARVESTER software [40], for the formation of reconstructed panmictic populations for the total number of pear genotypes (192 diploid and 118 triploid) and for those commercial varieties located in the Canary Islands (99 diploid and 66 triploid) for 12 and 18 SSRs; Table S1: Information on the pear samples used in this study: Code or accession name, origin, putative ploidy, genetic group and group assignment by structure analysis when K = 3 and 18 SSRs were considered; Table S2: SSRs used for molecular characterization: Locus, linkage group and PCR details.

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References

1. Food and Agriculture Organization of the United Nations. FAO Statistics Database on the World Wide. Available online: https://www.fao.org/faostat/es/#data/QCL (accessed on 27 June 2022).
2. Wu, J.; Wang, Y.; Xu, J.; Korban, S.S.; Fei, Z.; Tao, S.; Ming, R.; Tai, S.; Khan, A.M.; Postman, J.D.; et al. Diversification and Independent Domestication of Asian and European Pears. *Genome Biol.* **2018**, *19*, 77. [CrossRef] [PubMed]
3. Iglesias i Castellarnau, I.; Bonany Rocas, J.; Vilardell Coderch, P.; Ruiz, S. El Cultivo Del Peral En España. *Rev. Frutic.* **2015**, *45*, 6–33.
4. Ruiz de Galarreta Gómez, J.I.; Prohens, J.; Tierno, R. *Las Variedades Locales en la Mejora Genética de Plantas*; Departamento de Desarrollo Económico y Competitividad, Servicio Central de Publicaciones del Gobierno Vasco: Vitoria-Gasteiz, Spain, 2016; ISBN 9788445733950.
5. Deruwe, H. European Pear Crop Outlook 2018 a Quantitative and Qualitative Review. In Proceedings of the Prognosfruit, Warsaw, Poland, 8–10 August 2018.
6. Bao, L.; Chen, K.; Zhang, D.; Cao, Y.; Yamamoto, T.; Teng, Y. Genetic Diversity and Similarity of Pear (*Pyrus* L.) Cultivars Native to East Asia Revealed by SSR (Simple Sequence Repeat) Markers. *Genet. Resour. Crop Evol.* **2007**, *54*, 959–971. [CrossRef]
7. Miranda, C.; Urrestarazu, J.; Santesteban, L.G.; Royo, J.B.; Urbina, V. Genetic Diversity and Structure in a Collection of Ancient Spanish Pear Cultivars Assessed by Microsatellite Markers. *J. Am. Soc. Hortic. Sci.* **2010**, *135*, 428–437. [CrossRef]
8. dos Santos, A.R.F.; Ramos-Cabrer, A.M.; Díaz-Hernández, M.B.; Pereira-Lorenzo, S. Genetic Variability and Diversification Process in Local Pear Cultivars from Northwestern Spain Using Microsatellites. *Tree Genet. Genomes* **2011**, *7*, 1041–1056. [CrossRef]
9. Queiroz, Á.; Guimarães, J.B.; Sánchez, C.; Simões, F.; de Sousa, R.M.; Viegas, W.; Veloso, M.M. Genetic Diversity and Structure of the Portuguese Pear (*Pyrus communis* L.) Germplasm. *Sustainability* **2019**, *11*, 5340. [CrossRef]

10. Baccichet, I.; Foria, S.; Messina, R.; Peccol, E.; Losa, A.; Fabro, M.; Gori, G.; Zandigiacomo, P.; Cipriani, G.; Testolin, R. Genetic and Ploidy Diversity of Pear (*Pyrus* spp.) Germplasm of Friuli Venezia Giulia, Italy. *Genet. Resour. Crop Evol.* **2020**, *67*, 83–96. [CrossRef]
11. Alonso Segura, J.M.; Espiau Ramırez, M.T.; Pina Sobrino, A.; Rubio-Cabetas, M.J.; Fernández i Martì, A. Genetic Diversity of the Spanish Pear Germplasm Collection Assessed by SSRs. *Acta Hortic.* **2021**, *1303*, 37–44. [CrossRef]
12. Bielsa, F.J.; Irisarri, P.; Errea, P.; Pina, A. Genetic Diversity and Structure of Local Pear Cultivars from Mountainous Areas from Aragon (Northeastern Spain). *Agronomy* **2021**, *11*, 1778. [CrossRef]
13. Islam, M.; Ahmad, H.; Khalid, A.N.; Inamullah; Mohammad, K.; Masood, R.; Akhtar, N.; Afradi, S.G.; Ahmad, I. Pear (*Pyrus*): Genetic Diversity and Their Conservation. *Fresenius Environ. Bull.* **2021**, *30*, 2333–2342.
14. Sapkota, S.; Boggess, S.L.; Trigiano, R.N.; Klingeman, W.E.; Hadziabdic, D.; Coyle, D.R.; Nowicki, M. Microsatellite Loci Reveal High Genetic Diversity, Mutation, and Migration Rates as Invasion Drivers of Callery Pear (*Pyrus calleryana*) in the Southeastern United States. *Front. Genet.* **2022**, *13*, 861398. [CrossRef] [PubMed]
15. Instituto Nacional de Investigación y Tecnología Agraria. Inventario Nacional de Recursos Fitogenéticos. Available online: https://wwwx.inia.es/inventarionacional/Bus_genero.asp (accessed on 27 June 2022).
16. Bataillon, M. *La Isla de la Palma en 1561. Estampas Canarias de Juan Méndez Nieto*; Instituto de Estudios Canarios: San Cristóbal de La Laguna, Spain, 1987.
17. Frutuoso, G. *Las Islas Canarias (De "Saudade da Terra")*; Instituto de Estudios Canarios: San Cristóbal de La Laguna, Spain, 1964.
18. Gómez Gómez, M.A. *El Valle de Güímar en el Siglo XVI. Protocolos de Sancho de Urtarte*; Ayuntamiento de Güímar: Santa Cruz de Tenerife, Spain, 2000; ISBN 84-923966-5-2.
19. Afonso Morales, D.; Castro Martín, N.; González Díaz, A.J.; Lorenzo Rodríguez, R.; Medina Cabrera, C.E.; Monterrey Gutiérrez, A.B.; Morera Bello, M.E.; Ríos Mesa, D.J.; Tascón Rodríguez, C. *Variedades Agrícolas Tradicionales de Tenerife y la Palma*; ASAGA CANARIAS-AGRICOMAC: Santa Cruz de Tenerife, Spain, 2012.
20. Velázquez Barrera, M.E.; López Frías, R.; Méndez Hernández, C. *Medida del Frío Invernal en Tenerife en Base al Modelo Dinámico (Fishman et al., 1987)*; Servicio Técnico de Agricultura y Desarrollo Rural, Cabildo Insular de Tenerife: Santa Cruz de Tenerife, Spain, 2021.
21. García Brunton, J.; Pérez Tornero, O.; Cos Terrer, J.E.; Ruiz García, L.; Sánchez López, E. *Influencia del Cambio Climático en la Mejora Genética de Plantas*; Spanish Society of Horticultural Sciences, Spanish Society of Genetics: Murcia, Spain, 2018; ISBN 978-84-948233-8.
22. Pereira-Lorenzo, S.; Ramos-Cabrer, A.M.; Ferreira, V.; Díaz-Hernández, M.B.; Carnide, V.; Pinto-Carnide, O.; Rodrigues, R.; Velázquez-Barrera, M.E.; Rios-Mesa, D.; Ascasíbar-Errasti, J.; et al. Genetic Diversity and Core Collection of *Malus* × *domestica* in Northwestern Spain, Portugal and the Canary Islands by SSRs. *Sci. Hortic.* **2018**, *240*, 49–56. [CrossRef]
23. dos Santos, A.R.F. Diversidad Genética de La Colección de Peral Del Centro de Investigaciones Agrarias de Mabegondo (CIAM, Xunta de Galicia). Ph.D. Thesis, Universidad de Santiago de Compostela, Lugo, Spain, 2011.
24. Urrestarazu, J.; Royo, J.B.; Santesteban, L.G.; Miranda, C. Evaluating the Influence of the Microsatellite Marker Set on the Genetic Structure Inferred in *Pyrus communis* L. *PLoS ONE* **2015**, *10*, e0138417. [CrossRef]
25. Aubakirova, K.; Omasheva, M.; Ryabushkina, N.; Tazhibaev, T.; Kampitova, G.; Galiakparov, N. Evaluation of Five Protocols for DNA Extraction from Leaves of *Malus sieversii*, *Vitis vinifera*, and *Armeniaca vulgaris*. *Genet. Mol. Res.* **2014**, *13*, 1278–1287. [CrossRef]
26. Bokszczanin, K.; Przybyla, A.A. Copper (II) Acetate Improves the Quality of Pear (*Pyrus*) DNA during Extraction. *Plant Mol. Biol. Report.* **2006**, *24*, 249. [CrossRef]
27. Reija Abelairas, P.A. Estudio de la Diversidad Genética del Manzano Cultivado en el Oeste de la Península Ibérica y Canarias. Bachelor's Thesis, Universidad de Santiago de Compostela, Lugo, Spain, 2016.
28. Liebhard, R.; Gianfranceschi, L.; Koller, B.; Ryder, C.D.; Tarchini, R.; van de Weg, E.; Gessler, C. Development and Characterisation of 140 New Microsatellites in Apple (*Malus* x *Domestica* Borkh.). *Mol. Breed.* **2002**, *10*, 217–241. [CrossRef]
29. Vinatzer, B.A.; Patocchi, A.; Tartarini, S.; Gianfranceschi, L.; Sansavini, S.; Gessler, C. Isolation of Two Microsatellite Markers from BAC Clones of the Vf Scab Resistance Region and Molecular Characterization of Scab-Resistant Accessions in *Malus* Germplasm. *Plant Breed.* **2004**, *123*, 321–326. [CrossRef]
30. Gianfranceschi, L.; Seglias, N.; Tarchini, R.; Komjanc, M.; Gessler, C. Simple Sequence Repeats for the Genetic Analysis of Apple. *Theor. Appl. Genet.* **1998**, *96*, 1069–1076. [CrossRef]
31. Fernández-Fernández, F.; Harvey, N.G.; James, C.M. Isolation and Characterization of Polymorphic Microsatellite Markers from European Pear (*Pyrus communis* L.). *Mol. Ecol. Notes* **2006**, *6*, 1039–1041. [CrossRef]
32. Hokanson, S.C.; Szewc-Mcfadden, A.K.; Lamboy, W.F.; Mcferson, J.R.; Lamboy, W.F. Communicated by P. M. A. Tigerstedt Microsatellite (SSR) Markers Reveal Genetic Identities, Genetic Diversity and Relationships in a *Malus domestica* Borkh. Core Subset Collection. *Theor. Appl. Genet.* **1998**, *97*, 671–683. [CrossRef]
33. Evans, K.; Fernández-Fernández, F.; Govan, C. Harmonising Fingerprinting Protocols to Allow Comparisons between Germplasm Collections-*Pyrus*. *Acta Hortic.* **2009**, *814*, 103–106. [CrossRef]
34. Peakall, R.; Smouse, P.E. GENALEX 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Mol. Ecol. Notes* **2006**, *6*, 288–295. [CrossRef]

35. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
36. Lo, E.Y.; Stefanovic, S.; Dickinson, T.A. Population Genetic Structure of Diploid Sexual and Polyploid Apomictic Hawthorns (*Crataegus*; Rosaceae) in the Pacific Northwest. *Mol. Ecol.* **2009**, *18*, 1145–1160. [CrossRef] [PubMed]
37. Ferreira, V.; Ramos-Cabrer, A.M.; Carnide, V.; Pinto-Carnide, O.; Assunção, A.; Marreiros, A.; Rodrigues, R.; Pereira-Lorenzo, S.; Castro, I. Genetic Pool Structure of Local Apple Cultivars from Portugal Assessed by Microsatellites. *Tree Genet. Genomes* **2016**, *12*, 36. [CrossRef]
38. Pereira-Lorenzo, S.; Urrestarazu, J.; Ramos-Cabrer, A.M.; Miranda, C.; Pina, A.; Dapena, E.; Moreno, M.A.; Errea, P.; Llamero, N.; Díaz-Hernández, M.B.; et al. Analysis of the Genetic Diversity and Structure of the Spanish Apple Genetic Resources Suggests the Existence of an Iberian Genepool. *Ann. Appl. Biol.* **2017**, *171*, 424–440. [CrossRef]
39. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef]
40. Earl, D.A.; vonHoldt, B.M. STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
41. Breton, C.; Tersac, M.; Bervillé, A. Genetic Diversity and Gene Flow between the Wild Olive (Oleaster, *Olea europaea* L.) and the Olive: Several Pleistocene Refuge Zones in the Mediterranean Basin Suggested by Simple Sequence Repeats Analysis. *J. Biogeogr.* **2006**, *33*, 1916–1928. [CrossRef]
42. Pereira-Lorenzo, S.; Ramos-Cabrer, A.M.; González-Díaz, A.J.; Díaz-Hernández, M.B. Genetic Assessment of Local Apple Cultivars from La Palma, Spain, Using Simple Sequence Repeats (SSRs). *Sci. Hortic.* **2008**, *117*, 160–166. [CrossRef]
43. Pereira-Lorenzo, S.; Costa, R.M.L.; Ramos-Cabrer, A.M.; Ribeiro, C.A.M.; da Silva, M.F.S.; Manzano, G.; Barreneche, T. Variation in Grafted European Chestnut and Hybrids by Microsatellites Reveals Two Main Origins in the Iberian Peninsula. *Tree Genet. Genomes* **2010**, *6*, 701–715. [CrossRef]
44. Pereira-Lorenzo, S.; Costa, R.M.L.; Ramos-Cabrer, A.M.; Ciordia-Ara, M.; Ribeiro, C.A.M.; Borges, O.; Barreneche, T.; Donini, P. Chestnut Cultivar Diversification Process in the Iberian Peninsula, Canary Islands, and Azores. *Genome* **2011**, *54*, 301–315. [CrossRef] [PubMed]
45. Nei, M. Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. *Genetics* **1978**, *89*, 583–590. [CrossRef]
46. Meirmans, P.; van Tienderen, P. Genotype and Genodive: Two Programs for the Analysis of Genetic Diversity of Asexual Organisms. *Mol. Ecol. Notes* **2004**, *4*, 792–794. [CrossRef]
47. Excoffier, L.; Laval, G.; Schneider, S. Arlequin (Version 3.0): An Integrated Software Package for Population Genetics Data Analysis. *Evol. Bioinform. Online* **2005**, *1*, 47–50. [CrossRef]
48. Michdakis, Y.; Excoffied, L. A Generic Estimation of Population Subdivision Using Distances Between Alleles With Special Reference for Microsatellite Loci. *Genetics* **1996**, *142*, 1061–1064. [CrossRef] [PubMed]
49. Sokal, R.; Michener, C.A. A Statistical Method for Evaluating Systematic Relationships. *Univ. Kans. Sci. Bull.* **1958**, *38*, 1409–1438.
50. Rohlf, F.J. *NTSYS-Pc Version 2.2: Numerical Taxonomy and Multivariate Analysis System*; Exeter Software: New York, NY, USA, 2021; ISBN 0-925031-31-3.
51. Goodman, L.A. The Analysis of Multidimensional Contingency Tables When Some Variables Are Posterior to Others: A Modified Path Analysis Approach. *Biometrika* **1973**, *60*, 179–192. [CrossRef]
52. Fernández-Otero, C.I.; Ramos-Cabrer, A.M.; López-Díaz, J.E.; Pereira-Lorenzo, S. Evaluating the Diversity of Ecotypes of Red Clover (*Trifolium pratense* l.) from Northwestern Spain by Phenotypic Traits and Microsatellites. *Agronomy* **2021**, *11*, 2275. [CrossRef]
53. Pierantoni, L.; Cho, K.H.; Shin, L.S.; Chiodini, R.; Tartarini, S.; Dondini, L.; Kang, S.J.; Sansavini, S. Characterisation and Transferability of Apple SSRs to Two European Pear F1 Populations. *Theor. Appl. Genet.* **2004**, *109*, 1519–1524. [CrossRef]
54. Alessandri, S.; Cabrer, A.M.R.; Martìn, M.A.; Mattioni, C.; Pereira-Lorenzo, S.; Dondini, L. Genetic Characterization of Italian and Spanish Wild and Domesticated Chestnut Trees. *Sci. Hortic.* **2022**, *295*, 110882. [CrossRef]
55. Pérez, V.; Larrañaga, N.; Abdallah, D.; Wünsch, A.; Hormaza, J.I. Genetic Diversity of Local Peach (*Prunus persica*) Accessions from La Palma Island (Canary Islands, Spain). *Agronomy* **2020**, *10*, 457. [CrossRef]
56. Pereira-Lorenzo, S.; Belén Díaz-Hernández, M.; Ramos-Cabrer, A.M. Use of Highly Discriminating Morphological Characters and Isozymes in the Study of Spanish Chestnut Cultivars. *J. Am. Soc. Hortic. Sci.* **2006**, *131*, 770–779. [CrossRef]
57. Wambulwa, M.C.; Fan, P.Z.; Milne, R.; Wu, Z.Y.; Luo, Y.H.; Wang, Y.H.; Wang, H.; Gao, L.M.; Xiahou, Z.Y.; Jin, Y.C.; et al. Genetic Analysis of Walnut Cultivars from Southwest China: Implications for Germplasm Improvement. *Plant Divers.* **2021**. [CrossRef]
58. Ferradini, N.; Lancioni, H.; Torricelli, R.; Russi, L.; Ragione, I.D.; Cardinali, I.; Marconi, G.; Gramaccia, M.; Concezzi, L.; Achilli, A.; et al. Characterization and Phylogenetic Analysis of Ancient Italian Landraces of Pear. *Front. Plant Sci.* **2017**, *8*, 751. [CrossRef]
59. Queiroz, A.; Assunção, A.; Ramadas, I.; Viegas, W.; Veloso, M.M. Molecular Characterization of Portuguese Pear Landraces (*Pyrus communis* L.) Using SSR Markers. *Sci. Hortic.* **2015**, *183*, 72–76. [CrossRef]
60. Erez, A. *Temperate Fruit Crops in Warm Climates*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000.
61. Ministerio de Agricultura Pesca y Alimentación. Lista Española de Variedades Con Descripción Oficialmente Reconocida 2021. Available online: https://www.mapa.gob.es/es/agricultura/temas/medios-de-produccion/listadoespanoldevariedadescondorenero2021_tcm30-556166.pdf (accessed on 27 June 2022).

62. Viera y Clavijo, J. *Diccionario de Historia Natural de Las Islas Canarias. Tomo I*; Real Sociedad Económica Amigos del País: Las Palmas de Gran Canaria, Spain, 1866.
63. Hernández Hernández, J.M. *Cartas de Medianeros de Tenerife (1769–1893)*; Academia Canaria de la Lengua: San Cristóbal de La Laguna, Spain, 2003; ISBN 978-8-4960-5911-5.
64. Çoban, A. Genetic Characterization of Pear Cultivars (Pyrus communis) in Çoruh River Basin. Bachelor's Thesis, Middle East Technical University, Ankara, Turkey, 2019.
65. Bassil, N.; Hummer, K.E.; Postman, J.D.; Fazio, G.; Baldo, A.; Armas, I.; Williams, R. Nomenclature and Genetic Relationships of Apples and Pears from Terceira Island. *Genet. Resour. Crop Evol.* **2009**, *56*, 339–352. [CrossRef]
66. Sau, S.; Pastore, C.; D'hallewin, G.; Dondini, L.; Bacchetta, G. Characterisation of Microsatellite Loci in Sardinian Pears (*Pyrus communis* L. and *P. spinosa* Forssk.). *Sci. Hortic.* **2020**, *270*, 109443. [CrossRef]