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Genetic Characterization of a Plum Landrace Collection from La Palma, Canary Islands

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Abstract: A plum collection located in the island of La Palma, Canary Islands, composed of twenty-nine European and Japanese plums was analyzed using nine simple sequence repeat (SSR) highly polymorphic loci. First, a cytometry flow analysis was performed to determine the ploidy level. Sixteen accessions turned out diploid and thirteen hexaploid. According to morphological characteristics, fourteen of the sixteen diploid accessions were assigned to *Prunus salicina*, and two accessions to *P. cerasifera*. All the hexaploid accessions were assigned to *P. domestica*. The 29 accessions were compared using SSR markers with twenty-two *P. domestica* accessions maintained at the CITA plum germplasm collection located in Zaragoza, Aragón, Spain. A principal component analysis (PCA) and a clustering approach grouped the accessions according to the assigned species and geographical location, while some synonyms and homonyms were found within La Palma accessions. The two principal components explained 80.3% (67.3% and 13%, respectively) of the total variance. A tree generated with UPGMA hierarchical clustering and Bruvo distance grouped the accessions in two main clusters according to ploidy level and species assignment. The STRUCTURE approach clearly differentiated La Palma diploid accessions and some of the hexaploid accessions from those of the CITA collection. The results obtained could be used for management and conservation purposes of this valuable local plum germplasm.

Keywords: SSR; cytometry; germplasm; polyploidy; *Prunus*

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1. Introduction

Conservation of crop genetic resources is fundamental for future food security [1], since they are the main source for developing new varieties adapted to changing climatic conditions and with better qualities. However, in the last decades, an increase in genetic erosion in different crops caused by changes in land uses, introduction of more productive commercial varieties, the effects of climate change or the arrival of new diseases and pests has been observed. In order to maintain extant diversity, in situ and ex situ conservation strategies have been promoted [2]. In fruit trees, that are usually clonally propagated and where long-time seed conservation is generally difficult, ex situ germplasm cultivar collections are developed to guarantee the conservation of genetic diversity. Collections provide a genetic reservoir for growers and breeders, and an interesting plant material to be studied [2].

This global genetic erosion is even worse in particular situations in which the exchange of plant material is low, such as in the case of islands. One example is the island of La Palma, the northwesternmost island of the Canaries, part of the Macaronesia, a group of five volcanic North Atlantic archipelagos (Azores, Madeira, Savage Islands, Canary Islands and Cabo Verde), which extends from southwestern Europe to northwestern Africa. La Palma is a small island of 705 km^2 with elevations ranging from 0 to 2426 m above sea level (Figure 1). As the other Canary Islands, La Palma has a mild climate, without extreme temperatures, due to the influence of the north–northeast (NNE) trade winds and relatively cold ocean waters [3]. This climate favors the cultivation of temperate and subtropical fruit crop species such as peach, almond, plum, mango and avocado, as well as some tropical fruits at the sea level, such as bananas [4]. For centuries, isolation has favored local differentiation or crop diversity due to the limitation of gene flow. However, the crossroad geographical location of the Canary Islands has allowed vegetal material exchanges with other places, such as continental Europe or Central and South America. In fact, after the European arrival to America in 1492, La Palma became the most important harbor in the Canaries in the commercial route between mainland Europe and the New World. As a result, the island presents an interesting diversity of plant materials from diverse origins due to human migration or commercial transfers [5].

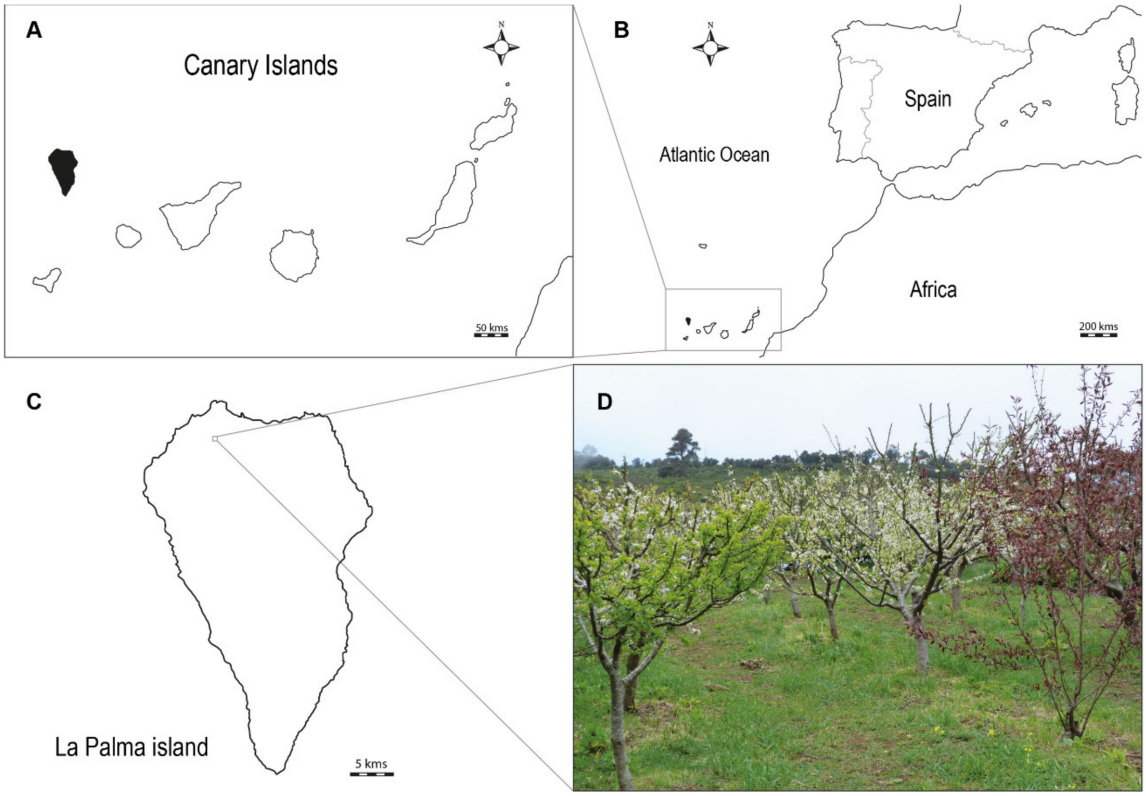

Figure 1. (**A**,**B**) Location of La Palma within the Canary Islands and in relation to mainland Spain; (**C**) location of Granja Experimental de Garafía-Cabildo Insular de La Palma plum collection; (**D**) photography of La Palma plum collection during the blooming season.

Agriculture is the largest source of wealth of La Palma. Since complex mechanization is difficult due to the mountainous nature of the island with a central volcano range, most agricultural production is restricted to small-scale farming in the coastal areas. At the coastal level, around 3000 ha are devoted to banana cultivation, which is mainly exported to continental Europe [4]. Other important crops are grapevine [6] and avocado [7]. In addition, many families have small orchards with fruit trees or vegetables for self-consumption and for sale in local markets. This small-scale agriculture has favored the

conservation of local varieties of different crops, which are being studied in the last few years, and the local La Palma authorities have dedicated important resources to preserve and characterize the threatened agronomic heritage of the island. Previous studies have revealed genetic relationships between La Palma local fruit trees with Spanish mainland landraces of almond [8], peach [9], apple [10], chestnut [11] or pear [12], but also exchange with different Latin American countries, mainly Cuba and Venezuela [13,14].

However, no detailed previous studies are available on the diversity of plums in La Palma. Plums comprise a large group of *Prunus* species, belonging to the family Rosaceae, with hundreds to thousands of years of domestication. Species of plums are mainly self-incompatible [15–18] with a gametophytic self-incompatibility (GSI) system as other *Prunus* species. GSI prevents fertilization of the ovules by their own pollen by arresting growth of incompatible pollen tubes in the style [19,20]. Chromosome numbers of plum species range from diploid to hexaploid. *P. salicina*, *P. americana*, *P. simonii* and *P. cerasifera* are diploid ($2n = 6x = 16$), *P. spinosa* is tetraploid ($2n = 6x = 32$) and *P. domestica* and *P. insititia* are hexaploid ($2n = 6x = 48$) [21]. Polyploidy is widespread in plants and is a mechanism of adaptation and speciation, which usually gives rise to more vigorous plants [22]. However, the complexity of the polyploid genome makes molecular studies more laborious [23,24]. Among plums, the most interesting species from an agronomic perspective are the European (*P. domestica*; $6x$) and the Japanese (*P. salicina*; $2x$) plums [25]. Plums have relevant economic importance and are consumed both as fresh or processed food, in jam, jellies, and for the production of spirits, with a world production over 12 million tons, half of which is concentrated in China [26]. They are grown mainly in Asia, Eastern Europe and South and North America. In Spain, production is over 153 thousand tons [26]. Several plum diversity studies have been carried out, mainly in diploid Japanese plum [27–29], but studies are scarcer in plum species with higher ploidy [24,30–36], probably due to the higher complexity of the analyses.

In order to preserve the genetic resources of plums in La Palma, an ex situ germplasm plum collection was created after a prospection of local cultivated varieties. In this work, we report the characterization of this collection through flow cytometry and molecular marker analysis with microsatellite markers. Both approaches have been widely used in the genus. Thus, ploidy level and cytogenetic studies have been used in *Prunus* for the characterization of cultivated and wild species [37–40]. On the other hand, microsatellite markers are one of the most used molecular markers for intraspecies characterization [41], due to advantages as their codominance, polymorphism, reproducibility and neutrality [42]. Microsatellites have been used previously in plums [24,27–36]. The availability of a European plum collection in mainland Spain (Zaragoza) allowed the comparison of the European plum genotypes conserved in La Palma with those of a broader germplasm collection. The collection in Zaragoza has a high number of "Reine Claude" accessions, including several of the type "Reine Claude Verte", which is the most appreciated cultivar in this group for its excellent organoleptic qualities, and is the most popular European plum cultivar in many regions of Europe.

2. Materials and Methods

2.1. Plant Material

A total of 51 plum accessions were analyzed in this study. Of those, twenty-nine adult plum trees (*P. domestica*, *P. cerasifera* and *P. salicina*) originated from a germplasm collection in the Granja Experimental de Garafía-Cabildo Insular de La Palma located in La Palma, Canary Islands (X:215870; Y:3190713; UTM:28) at 952 m above sea level. In addition, twenty-two European plums (*P. domestica*) were included, all of them [32] except "Stanley", "Ruth Gestteter", "Polinizador Zuera" and "Fraila" belonging to the Reine Claude type from the Spanish germplasm collection of CITA in Zaragoza (Spain).

2.2. Flow Cytometry Analysis

To better discriminate between the 29 diploid and hexaploid selected individuals of the La Palma plum collection, flow cytometry analysis was performed [39,40]. Young leaves were collected and put in plastic bags with moist cotton until ploidy was analyzed with flow cytometer following [43]. For that, the leaves were chopped (0.5 cm^2 pieces) with a sharp razor blade and deposited into a buffer solution designed for nuclei extraction (CyStain® UV Precise T Kit; Sysmex, Norderstedt, Germany). The crude solution was filtered with a nylon filter with a pore size of 30 µm and stained with an aqueous solution of 4′,6-diamidino-2-fenylindole. They were then analyzed with a Cyflow® PA flow cytometer (Sysmex), and DNA content was quantified relative to 2C DNA content from the tomato cultivar Moneymaker.

2.3. DNA Extraction and PCR Amplification

DNA extraction was performed from silica-gel-dried young leaves following a modified cetyltrimethylammonium bromide (CTAB) method for *Prunus* [44], using an extraction buffer with 350 mM Sorbitol, 100 mM Tris-HCl, 6.4 mM EDTA and 0.1% $NaHSO_3$ pH 7.5, and a nuclei lysis buffer with 200 mM Tris-HCl, 64 mM EDTA, 2 M NaCl, 2% CTAB and 5% N-lauryl sarcosine. Genomic DNA was amplified with nine SSR loci developed in peach and cherry, due to the transferability of these markers between *Prunus* species [45,46] and previously being used in European plum [32] (Table 1). PCR amplification was performed with 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.01% Tween-20, 20.3 mM $MgCl_2$, 0.1 mM of each dNTP, 0.3 µM of each primer, 20 ng of genomic DNA and 1 unit of BioTaq™ DNA polymerase (Bioline, London, UK) in a final volume of 15 µL. Forward primers were labelled with WellRed fluorescent dyes on the 5′ end (Sigma-Aldrich, St. Louis, MO, USA). Reactions were carried out on a thermocycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following temperature profile: an initial step of 2 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 57 °C, 1 min at 72 °C and a final step of 5 min at 72 °C. PCR products were separated by capillary electrophoresis on a CEQ™ 8000 capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA).

Table 1. SSR loci analyzed in this study.

SSR Locus	Forward	Reverse	Reference
BPPCT-002	TCGACAGCTTGATCTTGACC	CAATGCCTACGGAGATAAAAGAC	[47]
BPPCT-007	TCATTGCTCGTCATCAGC	CAGATTTCTGAAGTTAGCGGTA	[47]
BPPCT-010	AAAGCACAGCCCATAATGC	GTACTGTTACTGCTGGGAATGC	[47]
BPPCT-012	ACTTCCATTGTCAGGCATCA	GGAGCAACGATGGAGTGC	[47]
BPPCT-028	TCAAGTTAGCTGAGGATCGC	GAGCTTGCCTATGAGAAGACC	[47]
PMS3	TGGACTTCACTCATTTCAGAGA	ACTGCAGAGAATTTCACAACCA	[48]
UDP96-005	GTAACGCTCGCTACCACAAA	CACCCAGCTCATACACCTCA	[49]
UDP96-008	TTGTACACACCCTCAGCCTG	TGCTGAGGTTCAGGTGAGTG	[49]
UDP98-409	GCTGATGGGTTTTATGGTTTTC	CGGACTCTTATCCTATCAACA	[49]

2.4. Population Genetic Structure

SSR results were analyzed with the R package POLYSAT [50]. Samples were archived in three groups according to their origin and ploidy: diploid La Palma (16 accessions), with 14 *P. salicina* samples and two *P. cerasifera* from La Palma; hexaploid La Palma (13 accessions), with La Palma *P. domestica* landraces; and hexaploid CITA (22 accessions) with *P. domestica* accessions of the ex situ collection in mainland Spain, including 14 landraces and 8 commercial cultivars. Principal component analysis (PCA) based on pairwise Bruvo distance was performed and represented in R with the package factoextra [51]. In addition, a UPGMA dendrogram based on the pairwise Bruvo distance matrix was constructed, plotted with the same R package and edited with FigTree software [52]. POLYSAT data files were exported to STRUCTURE [53] and SPAGeDi [54] appropriate formats. The samples were ordered according to the UPGMA dendrogram results to run the Bayesian clustering algorithm implemented in STRUCTURE [53], using 20 interactions of 200,000 Markov

Chain Monte Carlo (MCMC) generations with a burn-in period of 20,000 generations [55] for K from 2 to 7. The interactions were combined using the large K greedy algorithm from the program CLUMPAK [56] that was used also for graphic representation.

2.5. Genetic Diversity Analyses

Genetic diversity indices for intra- and intergroups were calculated with SPAGeDi [54], which allows the analysis of polyploid species. The following parameters were estimated: number of alleles per locus (NA), average of effective number of alleles (Nae), expected heterozygosity (He), observed heterozygosity (Ho) and inbreeding coefficient (Fi). In addition, statistic pairwise indexes between groups were estimated with the ANOVA approach: measure of population differentiation (intraclass kinship coefficient) (Fst) [57], intraclass relatedness coefficient (Rho) [58] and Fst analogue based on allele size (Rst) [59,60].

3. Results

3.1. Species Discrimination by Ploidy Level

The flow cytometry results showed two ploidy levels in the 29 La Palma local plum samples analyzed; 16 accessions were diploid, and 13 accessions were hexaploid (Table 2); this and fruit and leaf phenotypic analyses (data not shown) allowed to establish that 14 accessions corresponded to *P. salicina* (2*n*), two to *P. cerasifera* (2*n*) and 13 to *P. domestica* (6*n*).

Table 2. List of 51 accessions used in this study from La Palma (LP) and CITA in Zaragoza (CITA) collections, with accession name, abbreviation name, locality of prospection, collection of origin, ploidy level analyzed using flow cytometry and assigned species.

Accession	Abbreviation	Locality	Collection	Ploidy	Species
Agustina negra 17 [1]	AN17	Puntagorda	LP	Diploid	*P. salicina*
Amarilla secona 57 [1]	AS57	Garafía	LP	Diploid	*P. salicina*
Blanca punta cumplida 11 [1]	BPC11	Garafía	LP	Diploid	*P. salicina*
Blanca japonesa 2 [1]	BJ2	Puntallana	LP	Diploid	*P. salicina*
Blanca japonesa 15 [1]	BJ15	Mazo	LP	Diploid	*P. salicina*
Blanca japonesa 21 [1]	BJ21	Breña Alta	LP	Diploid	*P. salicina*
De Catela 39 [1]	DC39	Garafía	LP	Diploid	*P. salicina*
Japonesa amarilla antigua 53 [1]	JAA53	Garafía	LP	Diploid	*P. salicina*
Morada 23 [1]	MR23	Puntallana	LP	Diploid	*P. cerasifera*
Morada 61 [1]	MR61	Garafía	LP	Diploid	*P. salicina*
Morada 70 [1]	MR70	Garafía	LP	Diploid	*P. cerasifera*
Negra1_35 [1]	N35	Puntallana	LP	Diploid	*P. salicina*
Negra2_45 [1]	N45	Puntallana	LP	Diploid	*P. salicina*
Pastosa 43 [1]	P43	Garafía	LP	Diploid	*P. salicina*
Roja 42 [1]	R42	Garafía	LP	Diploid	*P. salicina*
Rojizo 64 [1]	RZ64	Garafía	LP	Diploid	*P. salicina*
Agustina 10 [1]	A10	Garafía	LP	Hexaploid	*P. domestica*
Agustina 52 [1]	A52	Garafía	LP	Hexaploid	*P. domestica*
Agustina blanca 32 [1]	AB32	Puntagorda	LP	Hexaploid	*P. domestica*
Agustina blanca 50 [1]	AB50	Garafía	LP	Hexaploid	*P. domestica*
Blanca del país 38 [1]	BP38	Garafía	LP	Hexaploid	*P. domestica*
Huevo chivato 8 [1]	HC8	Garafía	LP	Hexaploid	*P. domestica*
Huevo chivato 19 [1]	HC19	Puntagorda	LP	Hexaploid	*P. domestica*
Huevo chivato 58 [1]	HC58	Garafía	LP	Hexaploid	*P. domestica*
Mulata 4 [1]	ML4	Garafía	LP	Hexaploid	*P. domestica*
Mulata 25 [1]	ML25	Garafía	LP	Hexaploid	*P. domestica*
Negra 5 [1]	N5	Garafía	LP	Hexaploid	*P. domestica*
Negra del país pequeña 67 [1]	NPP67	Tijarafe	LP	Hexaploid	*P. domestica*
Verde 27 [1]	V27	Garafía	LP	Hexaploid	*P. domestica*
RC Arandana [2]	RCA		CITA		*P. domestica*
RC Bavay [2]	RCB		CITA		*P. domestica*
RC Dorada [2]	RCD		CITA		*P. domestica*

Table 2. *Cont.*

Accession	Abbreviation	Locality	Collection	Ploidy	Species
RC Doullins [2]	RCDL		CITA		*P. domestica*
RC President [2]	RCP		CITA		*P. domestica*
RCV Alcor 1 [1]	RCVA1		CITA		*P. domestica*
RCV Alcor 2 [1]	RCVA2		CITA		*P. domestica*
RCV Aniñon [1]	RCVAN		CITA		*P. domestica*
RCV Arenal [1]	RCVAR		CITA		*P. domestica*
RCV Conde [1]	RCVC		CITA		*P. domestica*
RCV Domingo [1]	RCVD		CITA		*P. domestica*
RCV F-4 A-4 [1]	RCVF4		CITA		*P. domestica*
RCV F-9 A-10 [1]	RCVF9		CITA		*P. domestica*
RCV Puente Ave [1]	RCVPA		CITA		*P. domestica*
RCV Río Ribazo1 [1]	RCVR1		CITA		*P. domestica*
RCV Río Ribazo2 [1]	RCVR2		CITA		*P. domestica*
RCV Tobed [1]	RCVT		CITA		*P. domestica*
RCV Verde [2]	RCVV		CITA		*P. domestica*
Fraila [1]	FR		CITA		*P. domestica*
Polinizador Zuera [1]	PZ		CITA		*P. domestica*
Ruth Gestteter [2]	RG		CITA		*P. domestica*
Stanley [2]	STL		CITA		*P. domestica*

[1] Landrace; [2] commercial variety; RC = Reine Claude; RCV = Reine Claude Verte.

3.2. Genetic Grouping

In order to study the relation between the accessions and the initially established groups, a principal component analysis (PCA) was performed. The two principal components explained 80.3% (67.3% and 13%, respectively) of the total variance, allowing to clearly separate the two species according to ploidy level. The accessions of *P. salicina* were located on the left of the PCA figure (Figure 2), while accessions of *P. domestica* were located on the right. Many *P. domestica* accessions from La Palma, mainly "Agustina" and "Verde" types were overlapping with samples from the CITA collection. The two *P. cerasifera* samples, called "Morada", appeared at one end of the polygon that included all the diploid genotypes.

A tree generated with UPGMA hierarchical clustering and Bruvo distance grouped the accessions into two main clusters (Figure 3) according to ploidy level and species assignment. The first one included all the hexaploid accessions and could be subdivided into two groups: one with all the Reine Claude type *P. domestica* accessions from CITA and only one sample from La Palma, "Verde 27"; in this main group, the Reine Claude Verte accessions from CITA clustered together, except "RCV F-4 A-4" and "RCV Verde" that grouped together but with the other Reine Claude group. The second cluster grouped all the rest of the hexaploid *P. domestica* accessions from La Palma, and three samples from CITA ("Polinizador Zuera", "Stanley" and "Fraila") that are not "Reine Claude" types. All the La Palma diploid samples were placed in the second main group, which could be divided into two groups. One cluster included "Morada 61" and "Negra1_35", which are indistinguishable at the molecular level and probably are synonyms, and "Pastosa 43", "Agustina negra 17" and "de Catela 39". The second group included several synonymies under the common name of "Blanca japonesa", "Amarilla secona 57", "Rojizo 64", "Negra2_45", "Roja 42" and "Japonesa amarilla antigua 53". Among this group, the samples that showed the higher distance were the two accessions named Morada, "Morada 70" and "Morada 23", name given because of their purple leaves (*Morada* means "purple" in Spanish) and were considered as *P. cerasifera*.

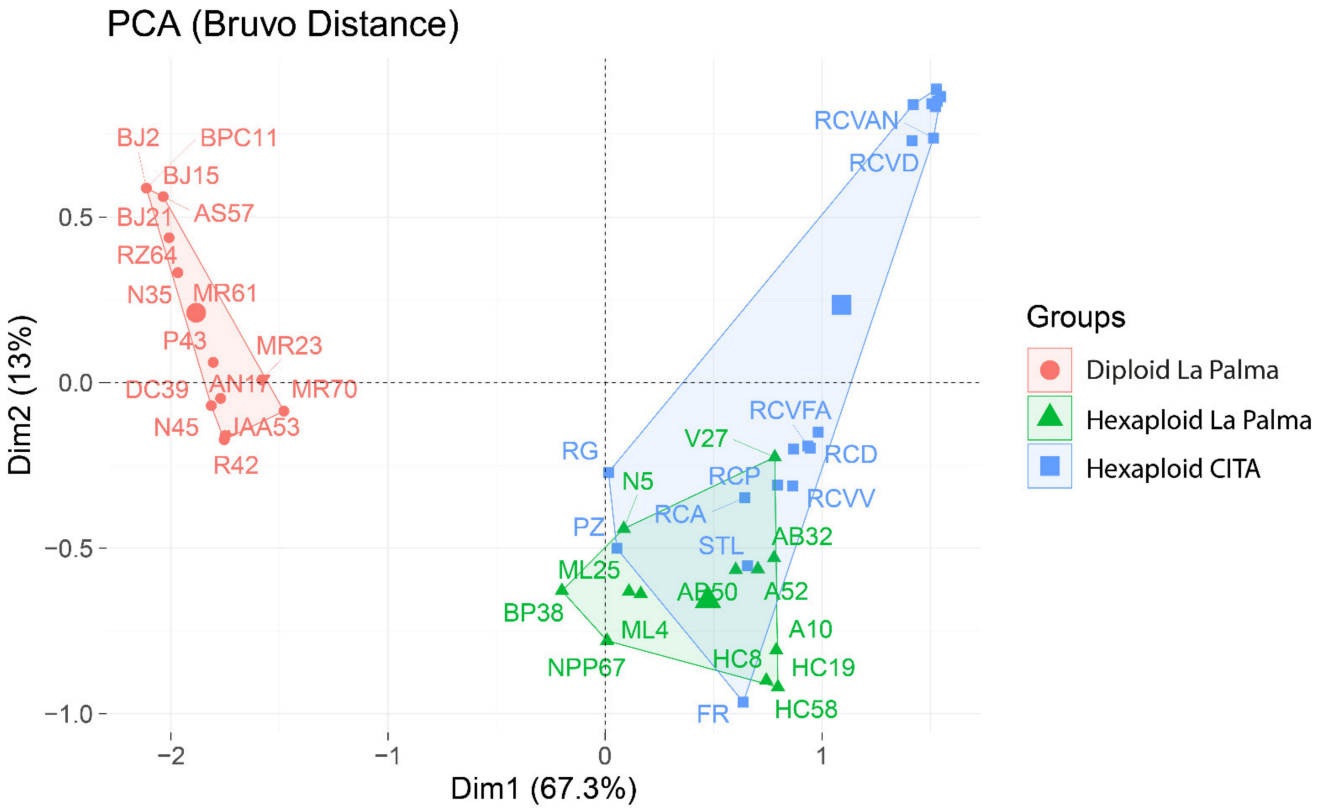

Figure 2. PCA based on Bruvo pairwise distance matrix. Diploid species (diploid LP plums) are in red. Hexaploid species of *Prunus domestica* are in green (hexaploid LP plums) and blue (hexaploid CITA plums). Accessions correspond to abbreviations in Table 1.

The STRUCTURE results considering $K = 3$ (colored bar in Figure 3) showed a clear differentiation between the hexaploid and diploid groups. However, in the hexaploid groups of *P. domestica*, the Reine Claude Verte accessions were separated. La Palma accessions and several accessions from CITA, with some Reine Claude (RC), grouped together.

When the K value increased ($K = 4$) (Figure 4), a new group appeared with only "Huevo chivato" accessions, which showed short Bruvo distances among them both in the dendrogram and PCA, and "Stanley" and "Polinizador Zuera". With $K = 5$, *P. domestica* accessions of La Palma were separated from those of the CITA collection, while for higher K values the groups remained similar.

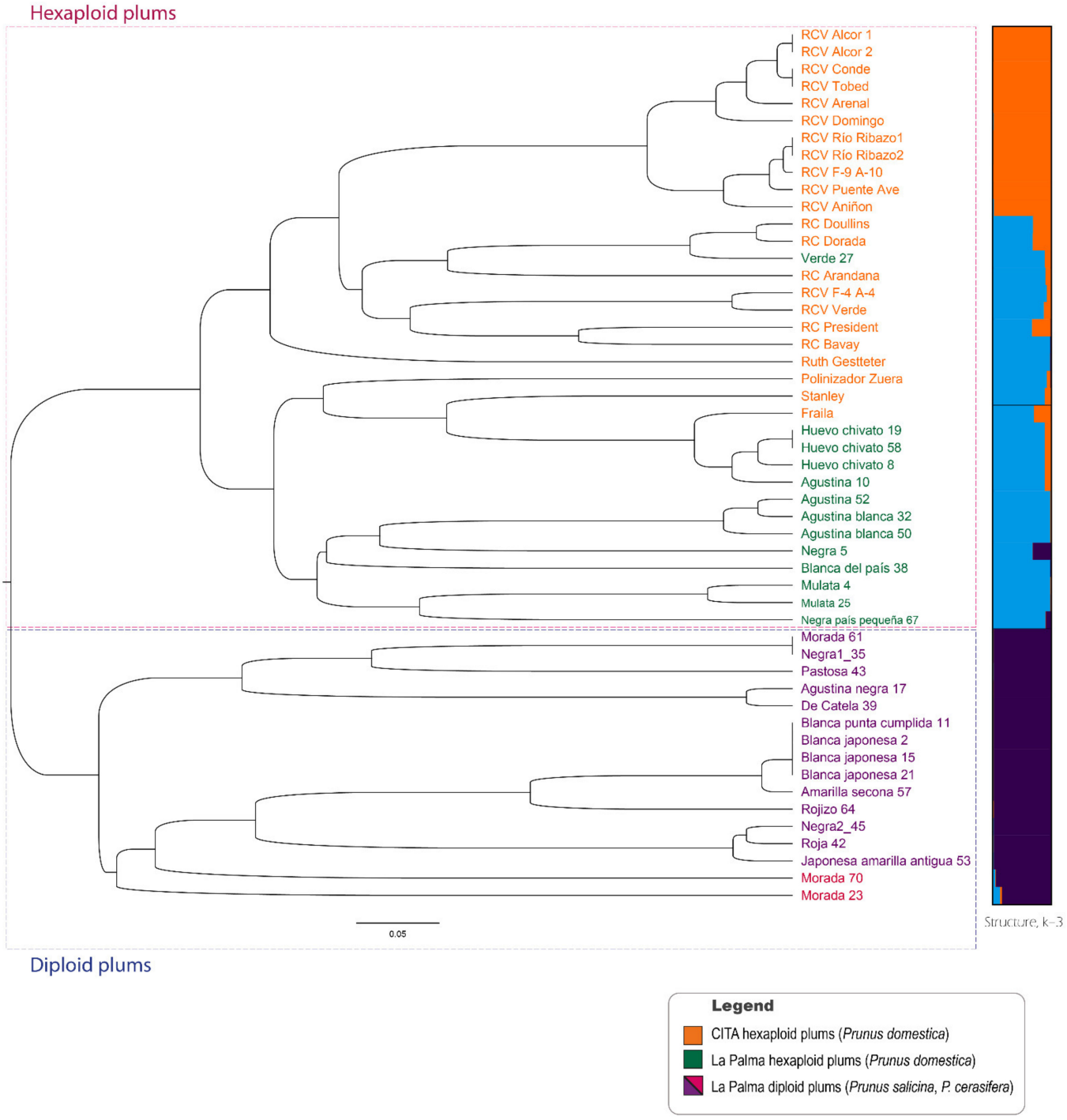

Figure 3. Bruvo-UPGMA cluster analysis based on nine SSR loci in plum species. Groups are indicated with colors: CITA hexaploid plums (*Prunus domestica*) in orange, La Palma hexaploid plums (*P. domestica*) in green and La Palma diploid plums (*P. salicina* and *P. cerasifera*) in purple and red, respectively. On the right, STRUCTURE results for $K = 3$ are shown.

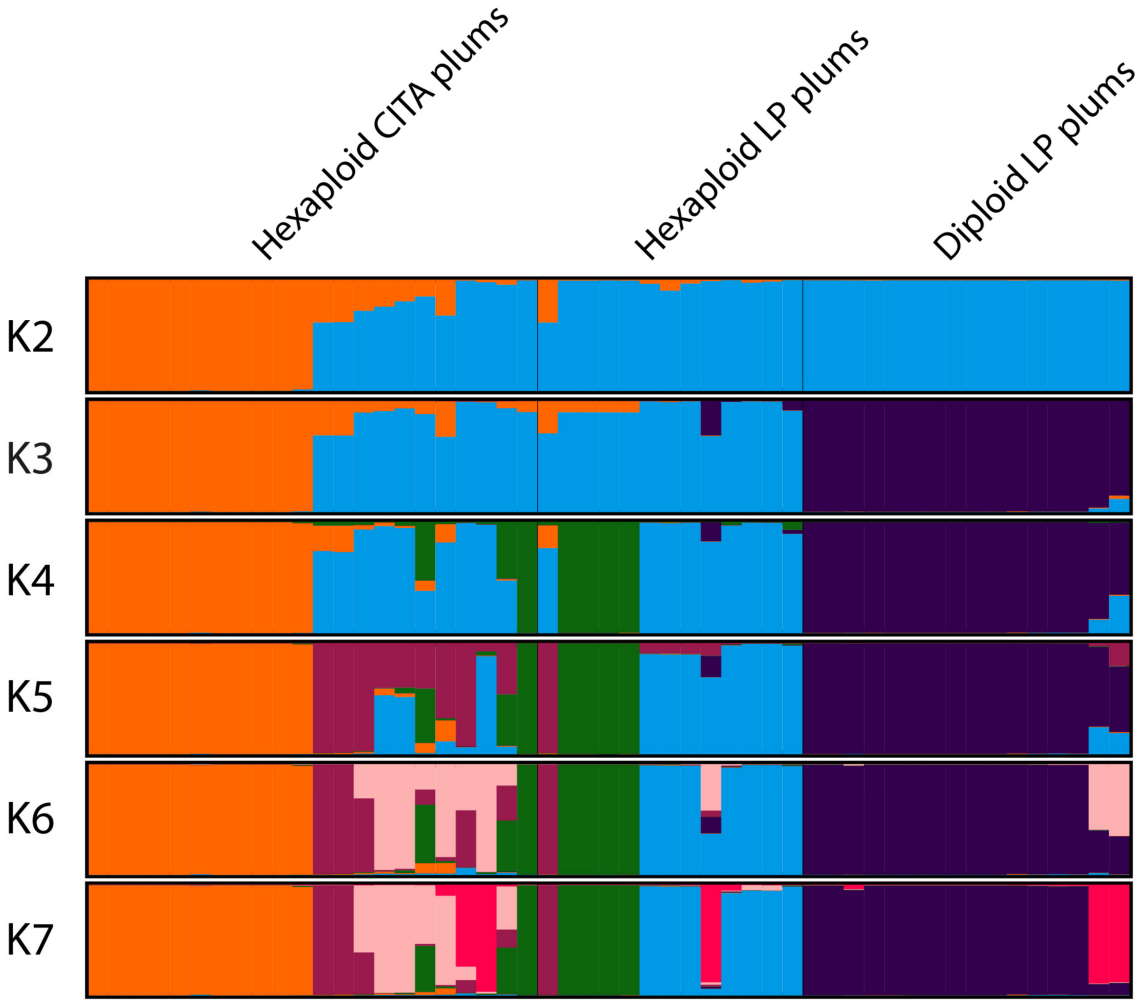

Figure 4. Bar plot of *Prunus* species inferred from Bayesian cluster STRUCTURE-CLUMPAK analyzed with Q = 20 repetitions for $K = 2$ to $K = 7$.

3.3. SSR Polymorphisms and Molecular Characterization

The average number of alleles per loci was the highest for the hexaploid CITA genotypes (10.56), followed by the hexaploid La Palma samples (9.33), and the lowest (6.89) for the diploid La Palma group (Table 3). However, the effective number of alleles was the highest for the hexaploid La Palma group (7.07), followed by the hexaploid CITA samples (5.91) and the diploid La Palma population (4.31). The expected heterozygosity was also the highest for the hexaploid La Palma group (0.85), followed by the hexaploid CITA samples (0.795) and the diploid La Palma accessions (0.76). However, the observed heterozygosity was the highest for the hexaploid CITA samples (0.935), followed by the diploid La Palma accessions (0.88) and the hexaploid La Palma samples (0.74). The Fi index was positive for La Palma accessions: 0.015 for diploid and 0.018 for hexaploidy plums, and negative (−0.027) for CITA samples. Statistics for pairwise Fst, Rho and Rst showed larger values between the hexaploid CITA and diploid La Palma groups, followed by the pair hexaploid and diploid La Palma accessions; the pair with the lowest differentiation was the two hexaploid groups (Table 4).

Table 3. Diversity indices for La Palma diploid accessions and La Palma and CITA hexaploid accessions. Number of alleles per locus (NA), average of effective number of alleles (NAe), expected heterozygosity (He), observed heterozygosity (Ho) and inbreeding coefficient (Fi).

		Diversity Index					
	n	**NA**	**NAe**	**AR**	**He**	**Ho**	**Fi**
Diploid La Palma	16	6.89	4.31	6.89	0.76	0.88	0.015
Hexaploid La Palma	13	9.33	7.07	8.55	0.85	0.74	0.018
Hexaploid CITA	22	10.56	5.91	8.18	0.795	0.935	−0.027

Table 4. Statistics for pairwise groups differences. Measure of population differentiation (intraclass kinship coefficient) (Fst), intraclass relatedness coefficient (Rho) and Fst analogue based on allele size (Rst), all with the ANOVA approach.

	Statistic for Pairwise								
	Fst			**Rho**			**Rst**		
	Dipl. LP	**Hex. LP**	**Hex. CITA**	**Dipl. LP**	**Hex. LP**	**Hex. CITA**	**Dipl. LP**	**Hex. LP**	**Hex. CITA**
Dipl. LP	-	0.166	0.220	-	0.346	0.469	-	0.045	0.116
Hex. LP	0.166	-	0.067	0.346	-	0.228	0.045	-	0.050
Hex. CITA	0.220	0.067	-	0.469	0.228	-	0.116	0.050	-

4. Discussion

4.1. Ploidy Level and Species Assignment

As a first step, the ploidy level of all the accessions from La Palma was analyzed, and the accessions were split in two groups: diploid and hexaploid. The diploid accessions were assigned to *P. salicina* (Japanese plum) and *P. cerasifera*, whereas the hexaploid accessions were assigned to *P. domestica* (European plum), taking also into account the morphological characteristics observed. Microsatellite markers (SSR) confirmed the ploidy level obtained by the cytometry assay and helped confirm the data and species taxonomy as shown in previous works [24,33]. Ploidies higher than 2*n* make the molecular analysis more complex [35], and the results are more ambiguous [34], hindering the interpretation of the multiple alleles per locus. In hexaploid species, a single locus can present up to six different alleles. When the number of alleles observed is lower than six, it is not possible, with the methodology used, to recognize those that are repeated. In any case, with the approach followed in this work, it was possible to unequivocally assign each of the accessions studied to its corresponding species.

4.2. Genetic Diversity and Population Structure

The PCA corroborated the differentiation between the two plum species, and a partial overlap was observed between the European plum accessions from La Palma and CITA. This mixture was also obtained with the STRUCTURE analysis for $K = 3$ and $K = 4$. These two groups were even more clearly separated in the dendrogram obtained with UPGMA using Bruvo distance. Only one accession from La Palma, "Verde 27", grouped with the CITA Reine Claude accessions and probably it could correspond to a Reine Claude type introduced from the Spain mainland. Previous studies of genetic diversity of temperate fruit crops in the Canary Islands, including La Palma, have established the origin of some of them, such as peach, apple or chestnut, in the Iberian Peninsula [9–11], so it is very likely that most of the plums of La Palma have a similar origin. However, in those works, some of the Canarian accessions showed some unique alleles compared to the accessions from the Iberian Peninsula, which could be due to sampling bias or to the introduction of genotypes from other origins [6,9], as it has been shown for other crops such as potato [13] or cauliflower [61].

The genetic indexes showed endogamy within La Palma plum samples in both hexaploid and diploid accessions. This is an expected result according to previous works in

other species, due to the small size and the isolation of the territory [9,61] and the reduced input of plant material. A lower inbreeding value was observed in the European plums from CITA, a collection that includes accessions from a larger geographical area. However, relatively low genetic diversity has been reported across cultivated plums in other regions [62].

4.3. Relations between La Palma Plum Accessions

Hexaploid samples from la Palma formed a group close to the group of the "Reine Claude" plums from the Spanish mainland collection, with the exception of "Verde 27", which grouped within the "Reine Claude" samples, suggesting that this could indeed be a Reine Claude type. In the hexaploid group of La Palma, three of the four plums from CITA that were not Reine Claude type, "Fraila", "Stanley" and "Polinizador Zuera" were included. The three accessions of "Huevo Chivato" showed zero or little distance among them (null alleles in two accessions for one locus UDP96-005), which could indicate a case of synonymy. This has also been found in other works with plums due to the graft propagation of interesting cultivars [34]. Several "Reine Claudia Verte" accessions from the CITA collection showed the same SSR profile although they could be distinguished at the morphological level [32]. In this same branch, an Agustina accession, "Agustina 10", was also present, whereas the rest of the samples of this variety grouped together in a nearby branch, a possible case of homonymy. The rest of La Palma hexaploid plums showed unique genotypes that allowed their unequivocal differentiation. It should be noted that the La Palma plum collection was made after intense ethnobotanical work, and only those trees that showed different morphological and pomological characteristics were conserved in the ex situ collection. Since the SSR markers and some accessions from the CITA collection were the same as those used by Gharbi et al. (2014) [32], the relation between the CITA accessions was similar to the one in that work, except that in our work, more accessions could be unequivocally discriminated. This can be explained by the fact that in Gharbi et al. (2014) [32] the separation of DNA amplification products was made by electrophoresis in metaphor agarose gels, while in this study, capillary electrophoresis, which offers better resolution, was used. Indeed, some of the "Reine Claude Verte" accessions that are synonyms are likely to have been vegetatively propagated, whereas those that are slightly different could represent seedlings.

In the diploid La Palma accessions, two main branches were obtained. In a first branch with five accessions, "Morada 61" and "Negra1_35" were indistinguishable, which could be a case of synonymy. Actually, the local name Morada, like the Morada used to assign *P. cerasifera* species, is a homonymy that produces confusion since *Morada* in Spanish refers to purple color, a frequent case in plum fruits. Other accessions present in this group include "Pastosa 43", "Agustina negra 17" and "De Catela 39". The local name Agustina is usually associated in La Palma with European plums, but in this case the accession is a Japanese plum. Plums of different geographic origins in the island named "Blanca japonesa" and "Blanca cumplida 11" were undistinguishable, probably indicating another case of synonymy.

4.4. Genetic Erosion and Conservation of La Palma Plums

This work contributes to improving the characterization of local La Palma plum accessions following previous works focused on the recovery of landraces, establishment, and maintenance of germplasm banks in other fruit tree crop species, such as almond [8], peach [9] or chestnut [11]. The combination of ploidy analyses with microsatellite molecular marker studies allowed to assign each accession to the corresponding species and study the diversity of the collection, revealing some synonyms and homonyms. Although the results showed low diversity, this collection is an important germplasm resource because the plums of La Palma are adapted to warm conditions, which could serve as a reservoir for plum breeders in the current context of global climate change. Small territories such as islands are threatened by genetic erosion [63] as a result of natural inbreeding or human

activities, such as changes in land use, increase in tourism or implementation of a few commercial new varieties, among others.

In this work, La Palma local plum accessions have been characterized using molecular markers. A comparison of La Palma samples with a Spanish local plum collection provided information about the relationship between them. The results showed that the most possible origin of most of the local plums from La Palma is the Iberian Peninsula. The abandonment of the cultivation of local landraces in La Palma and other islands in the Canaries makes its conservation necessary to preserve interesting genetic resources to optimize fruit production in the current context of climate change. The results obtained in this work will be relevant to optimize the conservation of plum genetic resources in La Palma, and similar works can be performed in other islands of the Macaronesia in order to preserve singular genotypes for future generations.

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