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Genetic Diversity and Structure of the Portuguese Pear (*Pyrus communis* L.) Germplasm

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Received: 12 July 2019; Accepted: 19 September 2019; Published: 27 September 2019

Abstract: A rich heritage of traditional pear varieties is kept in national Portuguese collections. Out of these varieties, "Rocha" dominates national pear production. Although a noticeable phenotypic variation among clones of this variety has been reported, little is known about its genetic variability, as to date molecular studies have been performed on a single "Rocha" clone. Eleven Simple Sequence Repeats (SSR) markers were used to assess the genetic diversity of 130 local cultivars, 80 of them being "Rocha" clones. The results allowed the differentiation of 75 genotypes of which 29 are "Rocha". Three synonyms groups and four homonymous groups of other local varieties were confirmed. A Bayesian model-based clustering approach identified two distinct clusters. Using flow cytometry, six cultivars were found to be triploids. These results show high genetic variability among "Rocha" clones. In conclusion, there is a need for different "Rocha" clones to be preserved to enable the correct selection of the multiplication material.

Keywords: pear genetic resources; SSR markers; identification; differentiation; clones discrimination

1. Introduction

Pear (*Pyrus communis* L.) is an important fruit crop that has long been cultivated in Europe. Portugal ranks third in pear production in Europe, after Italy and Spain [1]. A large number of local traditional varieties have been abandoned and replaced by modern varieties bred to meet the demands of both producers and consumers. However, from the heritage of traditional pear varieties, kept in Portuguese national collections [2], "Rocha" stands out as a true commercial success. The origin of this variety is uncertain but it is frequently assumed that it originated in the 19th century from a seedling tree detected by Mr. Rocha in his Sintra farm [3]. Due to the fruit high quality (taste, consistency, form, colour, high conservation capacity) "Rocha" was greatly propagated and cultivated in every orchard of "Região do Oeste" (northwest of Lisbon) [4]. In 1950, it ranked second among the pear varieties sold in Lisbon [5] and, since 2003, has been labeled as Protected Denomination of Origin. At present, "Rocha" orchards occupy about 11,000 ha with an average annual fruit production of 173,000 tons (99% of the national pear production). Around 60% of this production is exported to Brazil, United Kingdom,

France, Germany, and Morocco, with a revenue value that is expected to reach a value of 100 million € in 2019 [6].

A breeding program aiming at a "Rocha" clonal selection for yield and virus free plants began in 1970 and it was observed that a high clonal variation existed in farmers orchards. This fact prompted researchers of the Vieira de Natividade Fruit Research Station (Alcobaça) to establish a field collection of "Rocha" clones [4].

The genetic diversity of fruit trees is nowadays assessed using DNA-based molecular markers [7–12] and the Portuguese *Pyrus* diversity has already been studied using RAPDs [13], AFLPs [14] and microsatellites—SSRs [15]. SSRs have become markers of choice because they are highly informative, reliable, and easy to use for cultivar identification thereby improving the management of collections by enabling the identification of duplicates, synonymies, and homonymies [15–17]. They are also valuable tools to understand the origin of local varieties, and to ascertain the importance of introgression, polyploidy, and hybridization in their evolution [18].

In our previous SSR study, we targeted a limited number of Portuguese pear landraces [15]. The present study is extended to 12 more landraces that include "Rocha". Given the importance of "Rocha" and its high phenotypic variation, evident even in growth vigor and fruit size, the study also included 80 of its clones. The more reliable genetic evaluation with an increased sample number enables the understanding of evolutionary processes such as hybridization and polyploidy in the pear plants studied. The association between fruit size and ploidy level is also a valuable descriptor for characterization of plant genetic resources [19] and so, this diversity was evaluated in our accessions using microsatellite and genome size as markers. This work provides further insights into the diversity and genetic structure of pear landraces that contribute to improved management of this local resource.

2. Materials and Methods

2.1. Plant Material and DNA Extraction

A total of 132 accessions of *P. communis* (of which 50 are local varieties, 80 are "Rocha" clones, and two are the international references "Conference" and "Abbé Fétel") were analyzed in this study (Table 1). Forty (40) samples were collected from the Portuguese National Pear Collection, located at the Centro Experimental de Horto-Fruticultura, Quinta da Sobreira, Vidago (41° 38.2′ N, 7°34.6′ W) and at Quinta de Sergude, Felgueiras (41°22.7′N, 8°10.9′ W), twenty one (21) samples from the field collection from Instituto Nacional de Investigação Agrária e Veterinária (INIAV), at "Polo de Alcobaça", Alcobaça (39°57′28.90′′ N, 8°91′72.11′′ W) and seventy one (71) "Rocha" pear accessions from Quinta de São João, at Caldas da Rainha. The Quinta de S. João orchard was established in 1989. Accessions are referred in the text by their clone number and provenance (V for Vidago, S for Sergude, and A for Alcobaça); Rocha 9–Rocha 79 for pear accessions from Quinta de S. João orchard. DNA was isolated from young leaves using the innuPREP Plant DNA Kit (Analytik Jena AG, Berlin, Germany), according to the manufacturer's protocol. DNA quality was checked on 0.8% agarose gel, and the DNA concentration was estimated using a NanoDrop ND2000 spectrophotometer (Thermo Scientific, Massachusetts, MA, USA).

Table 1. *Pyrus communis* accessions characterized in the present study.

Reference	Local Designation	Field Collection
A1	ÁguaouAguinha	INIAV-Alcobaça
A2	Amendoa	INIAV-Alcobaça
V21	Amendoa	Quinta da Sobreira, Vidago
V04	Amendoa II	Quinta da Sobreira, Vidago
V12	Amendoa I	Quinta da Sobreira, Vidago
S15	Amorim	Quinta de Sergude, Felgueiras
V48	Amorim Branco	Quinta da Sobreira, Vidago
V39	Baguim	Quinta da Sobreira, Vidago

Table 1. *Cont.*

Reference	Local Designation	Field Collection
S05	Bela de Junho	Quinta de Sergude, Felgueiras
S11	Bela Feia 1	Quinta de Sergude, Felgueiras
V57	Bela Feia 2	Quinta da Sobreira, Vidago
S07	Bojarda	Quinta de Sergude, Felgueiras
V79	CabacinhaPrecoce	Quinta da Sobreira, Vidago
A3	CabeçaPequena	INIAV-Alcobaça
V115	Carapinheira	Quinta da Sobreira, Vidago
S12	CarapinheiraBranca	Quinta de Sergude, Felgueiras
A4	Carvalhal	INIAV-Alcobaça
V07	CoradaParda	Quinta da Sobreira, Vidago
V41	Coradinha	Quinta da Sobreira, Vidago
S14	Coxa de Freira	Quinta de Sergude, Felgueiras
A7	Cristo	INIAV-Alcobaça
V19	D. Joaquina	Quinta da Sobreira, Vidago
V80	Fim de Século	Quinta da Sobreira, Vidago
V28	Formiga	Quinta da Sobreira, Vidago
V97	LambeosDedos	Quinta da Sobreira, Vidago
A8	Marcelina	INIAV-Alcobaça
A9	Marmela	INIAV-Alcobaça
V13	Marmela 1	Quinta da Sobreira, Vidago
V89	Marmela 2	Quinta da Sobreira, Vidago
S18	Marquesinha	Quinta de Sergude, Felgueiras
V14	Nacional	Quinta da Sobreira, Vidago
A10	Pera Bonita	INIAV-Alcobaça
V26	PeraCabaça	Quinta da Sobreira, Vidago
V06	Pera de Inverno	Quinta da Sobreira, Vidago
A11	PeraMelão	INIAV-Alcobaça
A12	Perola	INIAV-Alcobaça
V16	PerolaAmarela	Quinta da Sobreira, Vidago
V66	PerolaAmarela 1	Quinta da Sobreira, Vidago
V75	PerolaAmarela 2	Quinta da Sobreira, Vidago
V63	PerolaBranca	Quinta da Sobreira, Vidago
V10	Pigarça	Quinta da Sobreira, Vidago
V95	Rabiça	Quinta da Sobreira, Vidago
V22	RaboTorto	Quinta da Sobreira, Vidago
V102	Rosa de Soure	Quinta da Sobreira, Vidago
V72	Rugosa	Quinta da Sobreira, Vidago
A14	S. Bartolomeu	INIAV-Alcobaça
V43	S. Bento de Chaves	Quinta da Sobreira, Vidago
A15	S. Crispim	INIAV-Alcobaça
V82	S. João de Silgueiros	Quinta da Sobreira, Vidago
V11	Tipo Cabaça	Quinta da Sobreira, Vidago
AF	Abbé Fétel	Quinta da Sobreira, Vidago
S19	Conference	Quinta de Sergude, Felgueiras
Rocha1—Rocha8	Pera Rocha	INIAV-Alcobaça
Rocha9—Rocha79	Pera Rocha	Quinta de S. João, Caldas da Rainha

Genome size and DNA ploidy levels were assessed using flow cytometry, performed by Plant Cytometry Services (www.PlantCytometry.nl). The determination of the nuclear DNA content of *Pyrus* was performed with the simultaneous analysis of nuclei isolated from *Pachysandra communis* and *P. communis*. The commercial variety "Conference" used as a control reference, is diploid (2X) and has a relative DNA ratio with *Pachysandra* of 0.37.

2.2. PCR Amplification and Fragments Sizing

The following eleven SSR loci, recommended by the European Cooperative Plant Genetic Resources (ECPGR), out of a list of 17, as the "minimum core" for pear fingerprinting [20] were used: CH01d08, CH03g07, CH01f07a, CH05c06, EMPc11, EMPc117, CH01d09, CH02b10, GD96, CH03d12, and CH04e03. Eight microsatellite loci were developed in apple [21] but present a high transferability and polymorphisms in the subtribe Pyrinae, allowing their use to assess cultivar identification in pear. The amplification was performed in a 10 µL solution using forward primers fluorescently labeled (D3 or D4) at the 5´ end and unlabeled reverse primers. PCR contained 2x QIAGEN Multiplex PCR Master Mix, 10x primer mix (2 µM of each primer), BSA BIOLABS (20 mg/mL), RNase-free water and 10 ng of genomic DNA. The PCR was programmed as follows: 15 min at 95 °C for initial denaturation, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at optimum Ta for 1 min and 30 s and extension at 72 °C for 1 min. A final extension step at 60 °C for 30 min and the reaction was finished with a continuous cycle at 8 °C. The reactions were conducted in an MWG AG Biotech thermocycler. The PCR reactions were carried out in two marker sets of 11 microsatellites, and mixtures of PCR products of different markers with different dyes (or distinct allele size ranges) were prepared for simultaneous detection of the amplified alleles. Subsequently, 1.0 µl of the PCR mixture was added to 24 µl formamide and 0.5 µl fragment size standard labeled with WellRED dye D1 (DNA size standard kit, 400, Beckman Coulter). Capillary electrophoresis was performed to separate the PCR products using the CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Brea, CA, USA). The sizes of the amplified products were determined based on a internal standard included with each sample. Data analysis was performed using the CEQ 8000 Fragment Analysis software, version 9.0, according to the manufacture´s recommendations (Beckman Coulter Inc., Brea, CA, USA). Sizes of fragments were automatically calculated using the CEQ 8000 Genetic Analysis System.

2.3. Data Analysis

GenAlEx 6.503 [22] was used to assess the genetic diversity measured as the number of alleles per locus (Na), observed and expected heterozygosity (Ho and He), number of private alleles and to calculate the pairwise standard genetic distances and the standard *Fst* (via frequency) values.

For diploid individuals, the genetic distance between each pair of individuals was calculated following Nei and Li [23]. Triploids were added to the analysis by hand calculation of their distances to the other individuals and by adding these to the distance matrix. The neighbor-joining algorithm, as implemented in the DARwin software package version 6.0.12 [24], was based on a dissimilarity matrix, and the reliability of the tree topology was assessed via bootstrapping over 1000 replicates.

Regarding the PCoA, the distance matrix was calculated following Peakall and Smouse [22]. Principal co-ordinate analysis (PCoA) was used to assess the diversity of all accessions but triploids.

The level of genetic stratification among the studied germplasm was assessed using the STRUCTURE v.2.3.4 software [25]. This analysis was performed based on the SSR genotypes with two alleles, excluding those genotypes for which a third allele was observed for one or more loci. The analysis was performed considering both the admixture model and the correlated allele frequencies between populations, with values of *K* set from 1 to 15. The population information was incorporated into the analyses (LOCPRIOR model). Each run consisted of a burn-in period of 10^4 steps followed by 10^6 MCMC (Monte Carlo Markov Chain) replicates assuming admixture model and correlated allele frequencies. K is the probable maximum population number that is assumed to represent and to contribute to the genotypes of sampled individuals. To check the consistency of the results between runs with the same K, fifteen replicates were run for each assumed K value. The approach suggested by Evanno et al. [26] was adopted to calculate the most likely value of K based on the second-order rate of change of the likelihood function with respect to K (DK). Once the number of genetic clusters was established, each individual was assigned to a cluster, and the overall membership of each sampled individual in the cluster was estimated.

3. Results

3.1. Overall Genetic Diversity

The eleven SSR loci amplified a total of 129 alleles with an average of 11.7 alleles ranging from eight (CH01d08 and GD96) to sixteen (CH01d09) and the average number of effective alleles was 5.8 (Table 2 and Table S1).

Table 2. Genetic diversity of 132 *P. communis* accessions as assessed using eleven SSRs loci. Na—number of alleles; Ne—effective number of alleles; Ho—observed heterozygosity; He—expected heterozygosity; PIC—polymorphism information content.

Locus	Na	Ne	Ho	He	PIC
CH01d08	8	4.114	0.766	0.757	0.657
CH01f07a	13	6.786	0.894	0.853	0.654
CH05c06	11	3.034	0.787	0.670	0.301
CH03g07	13	8.801	0.894	0.886	0.714
EMPc11	11	7.425	0.872	0.865	0.553
EMPc117	14	4.436	0.702	0.775	0.582
CH01d09	16	8.166	0.979	0.878	0.728
CH02b10	11	7.058	0.915	0.858	0.744
GD96	8	4.566	1.000	0.781	0.642
CH03d12	13	7.477	0.848	0.866	0.537
CH04e03	11	1.846	0.457	0.458	0.337
Average	11.72	5.79	0.83	0.79	0.59

The expected heterozygosity (He) ranged from 0.46 (CH04e03) to 0.89 (CH03g07) with an average of 0.79. The observed heterozygosity (Ho) ranged from 0.46 (CH04e03) to 1.00 (GD96) with an average of 0.83. The Polymorphism Information Content (PIC) value indicated that the SSR discriminatory power was quite good except for that of the CH05c06 and CH04e03, respectively 0.301 and 0.337 (Table 2).

The "Rocha" genotypes identified as synonymous were not considered for cluster analysis nor PCoA which means that the "Rocha" genotypes were therefore restricted to 29 (Table S2). The remaining traditional pear landraces correspond to 46 distinct SSR profiles (Table S1). Three groups of synonymies were identified (Table 3) and four groups of homonyms were detected (Table 4).

Table 3. Synonymies identified by SSR genotyping.

Synonymies (Name and Ref.)
"Amendoa I" (V12) = "RaboTorto" (V22)
"Amorim" (S15) = "Amorim Branco" (V48)
"Carapinheira" (V115) = "CarapinheiraBranca" (S12)

Table 4. Homonymies identified by SSR genotyping.

Homonymies (Name and Ref.)
"Amendoa" (A2); "Amendoa" (V21); "Amendoa II" (V04)
"Bela Feia 1" (S11); "Bela Feia 2" (V57)
"Marmela" (A9); "Marmela 1" (V13); "Marmela 2" (V89)
"PerolaAmarela" (V16); "PerolaAmarela1"(V66); "PerolaAmarela 2" (V75)

3.2. Genetic Relationships among Pear Genotypes

The neighbor joining dendrogram in the cluster analysis identified two main clusters. One cluster is entirely made of "Rocha" and includes 2 subgroups (Figure 1A,B).The other cluster is composed by 44 traditional pear landraces with distinct SSR profiles and two international references ("AF" and "S19"). "Amendoa I" (V12) and "RaboTorto" (V22), "Amorim" (S15), "Amorim Branco" (V48) and "LambeosDedos" (V97), "Carapinheira" (V115), and "CarapinheiraBranca" (S12) were undistinguished.

The landraces "Baguim" (V39), "CoradaParda" (V07), "Marquesinha" (S18), "PeraCabaça" (V26), and "PérolaAmarela 1" (V66) clustered with the remaining traditional cultivars. The landraces "Cristo" (A7) and "Bojarda" (S07) did not group with any of the two clusters forming two individual branches.

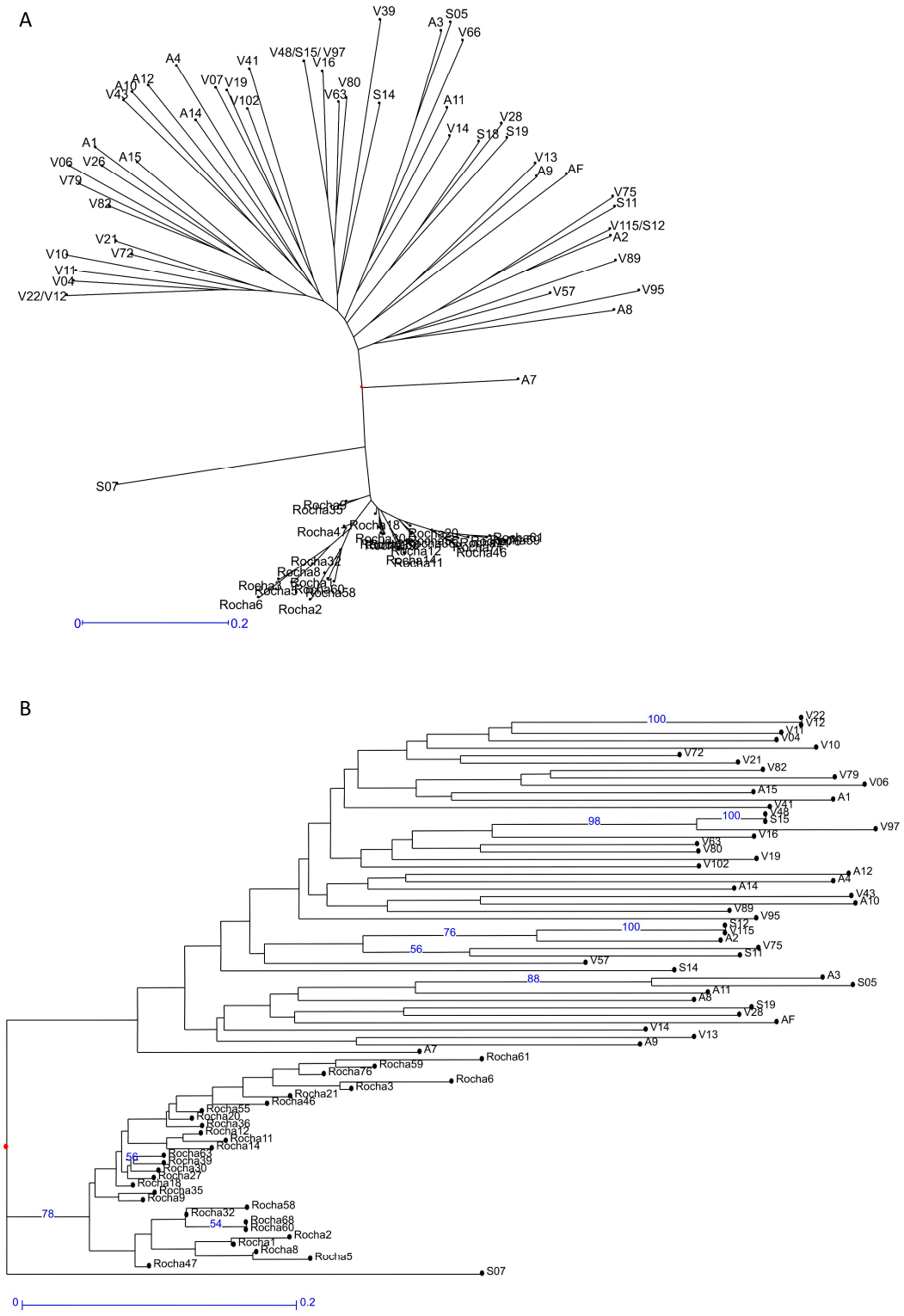

Figure 1. Neighbor joining dendrogram of *P. communis* genotypes identified by the eleven SSR markers used. (**A**)—Including triploid: "Baguim" (V39), "PérolaAmarela 1" (V66), "PeraCabaça" (V26), "CoradaParda" (V07), "Marquesinha" (S18); (**B**)—Not including the triploids but the bootstrap values (%) out of 1000 replications are indicated at the branches.

The overall PCoA analysis explained 45.94% of the variation and the first two axes accounted for 33.51% and 7.34% of the variation (Table 5). Despite the low percentage of variation, it is evident the presence of two main clusters. The landraces "Bojarda" (S07), "Cristo" (A7), "Marmela" (A9), and "Marmela 1" (V13) are outliers (Figure 2).

Table 5. Percentage of variation explained by the first three axes as a result of a principal co-ordinate analysis (PCoA).

Axes	1	2	3
%	33.51	7.34	5.09
Cum %	33.51	40.85	45.94

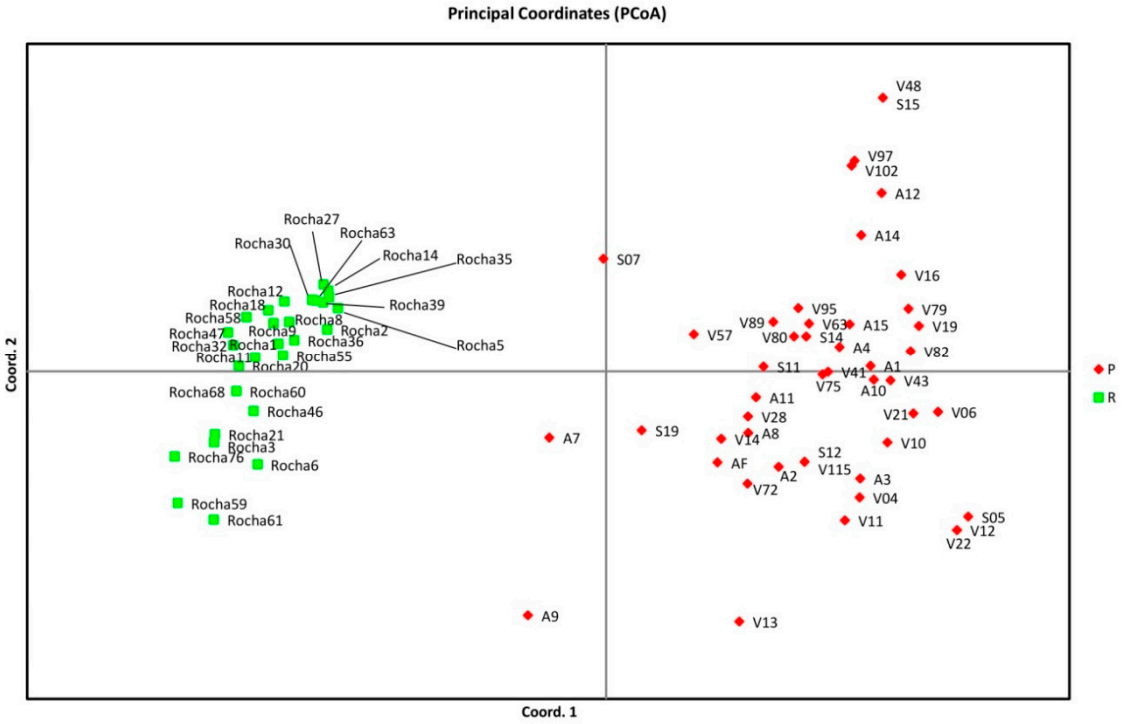

Figure 2. Principal co-ordinate analysis (PCoA) based on genetic distances showing two clustered populations. The groups are depicted using the color codes: Green—"Rocha" clones; Red—All the other pear landraces.

3.3. Genetic Structure of Pears

The Bayesian approach indicated that the most likely number of genetic clusters (hereafter called subpopulations) was K = 2 (Delta K = 569.68), while the second-best solution was K = 9 (Delta K = 1.99) (Figure 3 and Figure S1). At K = 2, all the 80 "Rocha" accessions were assigned to a unique sub-group (green) distinct from that subpopulation formed by the remaining 45 landraces plus the 2 international references (AF and S19) (red subpopulation). It is interesting to notice that the samples "Cristo" (A7), "Marmela" (A9), "Bojarda" (S07), and "Conference" (S19) have admixed characteristics (Figure 3A). At K = 9 it is still evident the existence of the two subpopulations, that of "Rocha" and that of the remaining germplasm accessions. However, in what concerns the remaining accessions there is a better definition of the differences existing between them. For instance, "Amorim Branco" (V48) and "LambeosDedos" (V97) are shown as undistinguished but "Cristo" (A7), "Marmela" (A9), and "Bojarda" (S07) are shown as partially admixed with "Rocha" (Figure 3B).

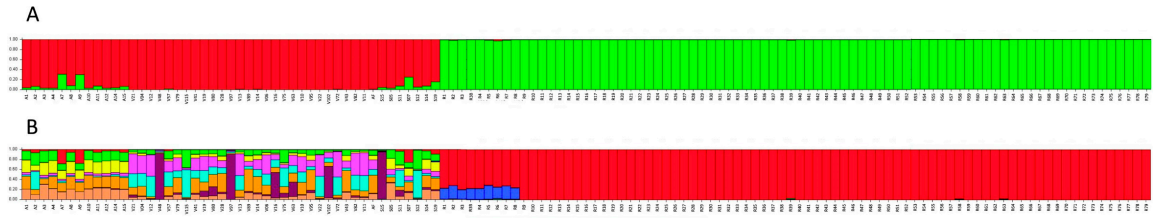

Figure 3. Graphical display of the results of the STRUCTURE analysis, inferred at $K = 2$ (**A**) and $K = 9$ (**B**). Each accession is represented by a vertical line segmented into a number of colors identical to the K number. The groups inferred at K = 2 are depicted in green ("Rocha" clones) and in red (all the other pear local cultivars). At K = 9 "Rocha" clones are depicted in red.

3.4. Polyploidy

Nuclear DNA content of the pear accessions indicated that "Baguim" (V39), "CoradaParda" (V07), "Marquesinha" (S18), "PeraCabaça" (V26), "PérolaAmarela 1" (V66), and "Rocha 25" are triploids (Figure S2). The remaining accessions are diploid.

4. Discussion

4.1. Overall Genetic Diversity

This study deepened the knowledge on diversity of Portuguese local pears, previously reported [15], since 130 accessions were now genotyped using eleven SSR markers. The number of alleles identified in our study was very similar to that referred to by Sehic et al. [27] except for the number of alleles produced by CH03d12 and CH04e3 loci. Mean heterozygosity values (Ho = 0.83 and He = 0.79) were similar to those found for local varieties and reported by other groups [18,27–30]. This high level of observed heterozygosity results from the fact that pear is an outcrossing plant and also has gametophytic self-incompatibility [31]. Ghosh et al. [32] found an observed heterozygosity equivalent to 0.63 and Hokanson et al. [33] reported 0.62 when nine SSR loci were used to study 142 Malus accessions. According to these studies, a correlation between heterozygosity and the plant pollination nature can be observed in the subfamily of Pomoideae.

The present study classified as synonymous "Amendoa I" (V12) and "RaboTorto" (V22) which have distinct phenotypic characteristics [34]. This was a result of mislabeling when the "RaboTorto" (V22) clone was sent to the National Collection (A. Assunção, personal communication).

4.2. Genetic Relationships among Pear Genotypes

In our study, the Portuguese pear accessions were clearly discriminated by the SSR markers. The 29 distinct "Rocha" genotypes clustered together in an independent genepool well separated from the cluster of the remaining landraces; "Bojarda" (S07) and "Cristo" (A07) are exceptions as they form separated branches (Figure 1). Minor genetic variation was observed among the different "Rocha" clones, which could be due to somatic mutations related to grafting [35] or, in some cases, the results of hybridization with unknown pear plants. Previously, a great variability and heterogeneity was referred among Malvasia grapevine clones that were cultivated in different geographic areas [36,37]. Clonal variation has also been reported within ancient Portuguese grapevine varieties [38]. It should be stressed that most of the reported molecular mechanisms underlying clonal variation are still unknown [35,36].

Contrary to our results, two previous work using AFLP and RAPD markers showed that "Rocha" clustered with the other Portuguese cultivars [13,14]. The authors of these works, besides using different molecular markers from ours, only analyzed one "Rocha" clone. This fact could explain the observed differences, taking in consideration that "Rocha" clones are highly variable. Bassil et al. [28], using SSRs to analyze the Portuguese pear genotypes, found that they were separated into two clades, with the only "Rocha" pear studied being placed between the two clades. Since this work only used

a "Rocha" accession, it is impossible to understand from such a study which is the "Rocha" relationship with the other Portuguese accessions.

When considering the characteristics of the Portuguese accessions other than "Rocha", it should be mentioned that the introduction of French, English and Belgian varieties in Portugal have played an important role in the genetic variability found in Portuguese pear genetic resources. For instance, it is known that "D. Joaquina" and "Amorim" (also called "Lambe-os-Dedos") did not originate in Portugal [14,15]. Also, Amaral [5] suggested that "Bojarda" could be of Italian or French origin.

4.3. Genetic Structure

At present, STRUCTURE is one of the most widely used population analysis methods to perform inferences of genetic structure in a set of samples and to understand the patterns of genetic diversity [12,39]. In our study, the STRUCTURE analysis allowed the clear distinction of the two subpopulations of "Rocha" from the old local varieties. The results can be obtained irrespective of the K value used, but at K = 9 we could, additionally, identify the origin of "Rocha" plants: Alcobaça (blue colour) and Caldas da Rainha (red colour) (Figure 3B). Since the analysis was performed with the "high priority" recommended marker sets [20], we consider that this result evidencing the two subpopulations for the genetic structure of the Portuguese pear is a robust one. In fact, Urrestarazu et al. [12] confirmed the ability of STRUCTURE to detect a clear differentiation of *P. communis* germplasm and suggested that several genepools could be considered. Also using STRUCTURE, Santos et al. [18] found a division between Northern European cultivars and Western Spanish pear germplasm. Independent and local selection was also reported for other fruit trees such as Western Mediterranean olive cultivars [40,41].

When we performed a PCoA analysis, we obtained results that were in accordance with the results of STRUCTURE, where two main subpopulations are visible. Concerning the accessions "Cristo" (A7), "Marmela" (A9), and "Bojarda" (S07), which in the PCoA graphic are in an intermediate position between the two subpopulations (Figure 2), it is not possible to know their origin for certain although Amaral [5] suggested that S07 could be of Italian or French origin.

4.4. Polyploidy

The majority of cultivated pears are diploid (2n = 2X = 34) but a few cultivars of *P. communis* are polyploids [28,42] and it is known that the size of the *Pyrus* genome was influenced by polyploidy [43]. Queiroz et al. [15], using six SSRs, verified that "Baguim" (V39), "PerolaAmarela 1" (V66), and "PeraCabaça" (V26) were triploids, which we confirmed in the present work using flow cytometry. Furthermore, this technique also identified "CoradaParda" (V07), "Marquesinha" (S18), and "Rocha 25" as triploids. These "Rocha" polyploidy clones are from a field collection that resulted from clonal selection performed in different farmers´ fields, on the basis of important agronomic traits such as yield, fruit size, and virus free plants. Despite the agronomic importance of fruit size, it was not possible to directly correlate it with a ploidy level.

5. Conclusions

Our results provide useful information for improved management and conservation of the *P. communis* germplasm in Portugal. "Rocha" has a relatively independent genepool with significant intra-variety diversity. This suggests the need to preserve different "Rocha" clones for the correct selection of multiplication material. Given the importance of polyploidy in crop improvement, more research is needed to understand its association with better agronomic performance that includes fruit size.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/11/19/5340/s1, Table S1: SSR screening of 132 P. communis accessions using 11 loci. Table S2: The 29 "Rocha" accessions that were used for cluster analysis and PCoA analysis. Figure S1: Exploration of K values for STRUCTURE analysis of pear germplasm. Figure S2: Flow cytometric histograms of relative fluorescence intensities of stained nuclei isolated from P. communis.

Author Contributions: M.M.V. and Á.Q. conceived the study; R.M.d.S. selected and provided the germplasm from the INIAV collection; F.S. participated in sample collecting; J.B.G. and Á.Q. performed the lab work and the SSR data analysis; Á.Q., C.S. and M.M.V. wrote the manuscript; W.V. critically revised the manuscript. All authors read and approved the final manuscript.

Funding: This study was supported by the project InovPomo—"Melhoramento do processo produtivo de peras e maçãs através da conservação e caracterização do material vegetal" (Proder 40448) and by Fundação para a Ciência e a Tecnologia (UID/AGR/04129/2019).

Acknowledgments: We are very grateful to Luis Carneiro for help in statistical analysis and A. Assunção for providing the pear samples from the Portuguese National Pear Collection.

Conflicts of Interest: The authors declare no conflict of interest.

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