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Characterisation of Microsatellite Loci in Sardinian Pears (*Pyrus communis* L. and *P. spinosa* Forssk.).

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Abstract

The Sardinian pear germplasm is an important resource of genetic diversity that can be used for increasing data on European pear germplasm, to optimise the efficiency of the association studies within the genome and to identify genomic regions that control the main horticultural traits. A set of 109 Sardinian pear genotypes, composed of 82 *Pyrus communis* cultivars, 24 wild populations of *P. spinosa* and three international reference cultivars, was analysed using nine SSR markers to assess the genetic diversity of Sardinian pears, determine their genetic structure and study the cases of synonymies and homonymies. The comparison of SSR profiles indicated four groups of diploid accessions with the same SSR profile. The alignment with the Sardinian dataset, pointed out a clear genotype distinctiveness. For all studied SSR loci, 15 specific rare alleles were identified, with a minimum of two alleles found in the database of analysed accessions for the SSRs EMPc11 and EMPc117. The overall allelic diversity revealed a high polymorphism in the analysed Sardinian germplasm. The structure analysis allowed us to identify four gene pool groups (Sardinian cultivars, Japanese cultivars, late-ripening cultivars and, the most famous, standard cultivars). These results were confirmed by Evanno's $\triangle k$ statistical

analysis which has shown unequivocally that k = 4 (△k = 150) is the most likely stratification level of the cluster. The Q values of *P. communis* and *P. spinosa* accessions have confirmed an allelic interchange between wild and cultivated genotypes.

Keywords: Sardinia; pear; molecular marker; genetic structure.

1. Introduction

The husbandry of pear trees in Sardinia has a long history, which involves domestication processes of autochthonous species (*Pyrus communis* L. and *P. spinosa* Forssk.). Indeed, literature dates back to Aristotle (384-322 BC), who exalted the island of Ichnusa (actual Sardinia) for its fruits, and to a description of 39 pear cultivars by Pliny (AD 23-79). Then, particularities of pear cultivars were reported in the scripts of Pausania, Polibio and Diodoro (Agabbio, 1994). In Sardinia, over a hundred pear accessions with different morphological characteristics of local origin have been recently examined, highlighting the literature on the rich heritage of ancient cultivars (Agabbio et al., 1986, 2015). Still, lifestyle changes and the, agronomic and productive characteristics of new breeds foster genetic erosion of these ancient cultivars in that they do not ensure comparable income with other economic activities (Muresu et al., 1997). However, in the present scenario, rather than the sole economic aspect, evidence is provided evermore on the key value of genetic diversity. Indeed, ancient local cultivars own a gene pool gathered and preserved over years under a broad range of different environmental conditions (Hammer et al., 2003). Since current breeding programs have not yet exploited this high variability for many traits, as already in progress for apple and other species, shedding light on Sardinian *Pyrus* genotype distinctiveness is pivotal (Liang et al., 2015).

Guided breeding programs start from an efficient molecular characterisation of known cultivars aimed at managing the genetic heritage by identifying clonal relationships, synonyms and homonyms. In fact, the assessment of genetic and phenotypic diversity is the starting point of screening germplasm collections for valuable characteristics useful in breeding programs. Following the marker era take-off, an increasing number of papers described the relationships among pear cultivars by using molecular markers: RFLPs (Iketani et al., 1998), RAPDs (Oliveira et al., 1999), and AFLPs (Monte-Corvo et al., 2000; Dolatowski et al., 2004; Bao et al., 2008). In eukaryotic genomes, microsatellites or simple sequence repetitions (SSR) are ubiquitous and easily employed as markers for both animal and plant species. Therefore, these approaches are highly reproducible and are of multi-allelic nature, codominant inheritance, and relative abundance and provide good genome coverage (Weber and Mat, 1989; Powell et al., 1996).

In recent years, main investigations on SSR primers of the genus *Pyrus* L. have focused on the varietal heritage of the Japanese *P. pyrifolia* Nak., and several studies were also conducted on the European *P. communis* (Yamamoto et al., 2002

59 a, b; Fernández-Fernández et al., 2006; Bao et al., 2007). Evans et al. (2007) standardised some SSRs markers useful to
60 compare *P. communis* accessions within European germplasm collections. Genetic diversity analysis in pear by using SSR
61 markers is widely reported in literature and then successfully used in Italy (Martinelli et al., 2009; Ferradini et al., 2017;
62 Bennici et al., 2018, Baccichet et al., 2020), Spain (Miranda et al., 2010; Ferreira dos Santos et al., 2011; Urrestarazu et al.,
63 2015), Germany and Romania (Puskas et al 2015), Portugal (Queiroz et al., 2015, 2019), Bosnia (Gasi et al., 2013), and
64 Slovenia (Sisko et al., 2009). Additional noteworthy characterisations of pear genetic variability were performed in Europe
65 (Wünsch and Hormaza, 2007; Sehic et al., 2012), in Africa (Tunisia: Brini et al., 2008; Ouni et al 2020) and in Asia (Yue
66 et al., 2018), including some specific Asian regions (Ahmed et al., 2010; Rana et al., 2015).
67 In Italy, recent characterisation of the Mount Etna pear germplasm aimed to introduce specific traits into breeding
68 programmes by exploiting genetic variability related to drought resistance, low chilly unit requirement, adaptation to daily
69 temperature fluctuation and low pest and disease incidence (Bennici et al., 2018).
70 At present, a set of useful SSR markers for pear genetic screening has been approved by the European Cooperative
71 Program for Plant Genetic Resources (ECPGR), and following the successful use of screening pear accessions of the
72 Brogdale National Fruit Collection (NFC) (Fernández-Fernández, 2010), a complete dataset is now available at the Institute
73 website (http://www.emr.ac.uk/SPFeliFernandez.htm). The availability of datasets describing the allelic pattern of hundreds
74 of accessions combined with the use of common sets of markers, was employed to define gene flows among local
75 germplasms of *Malus domestica* Borkh. (Urrestarazu et al., 2016). The same approach provided evidence of allelic
76 interchange between wild populations and cultivated pear germplasm of the Mount Etna (Bennici et al., 2018). This result
77 supports previous findings on Iranian *P. communis* germplasm genotypes, where gene transfer was highlighted by the
78 presence of new S-alleles possibly introduced from other domesticated or wild pear species as corroborated by plastidial
79 DNA analysis of the same genotypes (Gharehaghaji et al., 2014a). According to Gharehaghaji et al. (2014b), these results
80 and the cross-compatibility of wild and domesticated *Pyrus* taxa might have left a genetic trace on the traditional Iranian
81 pear germplasm. These interesting findings along with the great number of Sardinian pear genotypes, both domesticated
82 and wild ones, are the catalyst of the present work. This research aims to, first estimate the genetic relationship between
83 wild and local cultivated germplasm pear accessions and, second, extend this investigation to nationally and internationally
84 known cultivars. The outcomes will shed light on potential flows among the analysed gene pools. To reach this goal, the
85 constructed allele dataset of Sardinian germplasm pear accessions will be aligned with the one reported by Sehic et al.
86 (2012). This approach aims to increase *Pyrus* molecular marker datasets and sheds light on other homonymous and/or
87 synonymous genotypes difficult to distinguish using standard morphological descriptors.
88
89

90 **2. Materials and Methods**

91 *2.1. Germplasm material, DNA extraction and SSR genotyping*

92 The list of 108 analysed accessions is provided in Table 1. This inventory includes 76 *P. communis* cultivars from the
93 Sardinian germplasm conserved in the CNR-ISPA catalogue field (39°56'04.2" N and 8°35'46" E, 7 m a.s.l.), 5 from the
94 Fo.Re.S.T.A.S catalogue field (40°25'55" N and 9°01'43" E, 540 m a.s.l.), 24 representative accessions of *P. spinosa*
95 collected all over Sardinia and three international reference cultivars ('Williams', 'Abate' and 'Keiser'). *P. communis*
96 cultivars reported in this list and used throughout the work are those given by local communities and adopted in the
97 catalogue fields and, for *P. spinosa,* the geographical area where the mother plants are localised.

98 For each of them, genomic DNA was extracted from 50 mg of young freeze-dried leaves following the standard CTAB
99 protocol (Maguire et al., 1994). Genomic DNA was quantified by Nanodrop™ ND-1000 Spectrophotometer (Thermo
100 Scientific, Wilmington, DE, USA) and diluted to 10 ng/µL.

101 A set of nine SSRs primers (CH01d09, CH02b10, CH01f07a, CH03d12, CH04e03, CH05c06, EMPc11, EMPc117 and
102 GD147), chosen among the ECPGR approved ones, was used for pear genotyping (Evans et al., 2007; Sehic et al., 2012).
103 Primer sequences are also available on the HiDRAS (High-quality Disease Resistant Apples for Sustainable Agriculture)
104 website (http://users.unimi.it/hidras/). Forward SSR primers were labelled with FAM or HEX fluorescent dyes. The nine
105 SSR markers used for the analyses were selected according to the work carried out by Sehic et al. (2012), in which ten
106 apple markers for pears were used. The high transferability of SSRs among related species, such as apple and pear, was
107 abundantly demonstrated (since Pierantoni et al., 2004).

108 The amplification of markers was performed using a PCR protocol for apple as described by Liang et al. (2015). In
109 short, PCR was performed in a 10 µL reaction mixture containing 1× reaction buffer (Applied Biosystems, Foster City, CA,
110 USA), 0.6 mM $MgCl_2$, 0.4 mM dNTPs (Fermentas, Lithuania), 1 nM of each SSR locus-specific primer, 0.125 µL Taq
111 (5u/µL, Fisher Molecular Biology, Rome, Italy) and 10 ng genomic DNA.

112 PCR reactions were carried out in a 2720 thermal cycler (Applied Biosystems) with the following amplification
113 protocol: initial denaturation step at 94°C for three min, followed by 32 cycles at 94°C for 30 sec, at 58°C for one min and
114 at 72°C for one min and a final extension step at 72°C for 10 min.

115 Multi-pooling groups (MPG) of SSRs labelled with the two different fluorescent dyes were designed for SSR
116 genotyping on an ABI PRISM 3730 DNA analyser. SSRs were pooled by mixing PCR products labelled with different
117 dyes in a ratio of 1:1 for HEX:FAM; 3µl of the PCR products mixture was added to 7 µl of formamide containing 0.2 µL
118 of GeneScan 500 LIZ size standard (Applied Biosystems). Fragments were visually analysed and scored using Peak Scanner
119 v.1.0 (Applied Biosystems). To monitor the reproducibility in different amplifications, three reference cultivars ('Abate',
120 'Kaiser' and 'William', were included in each single run.

121

122 *2.2. SSR polymorphism, cluster and structure analysis*

123 SSR profiles were used to obtain a large dataset for the 109 pear genotypes used in this study. The collected data were
124 organised in a square matrix in which code '0' and '1' were used for allele absence and presence, respectively (code for missing
125 data was 9).

126 The genetic distance between cultivar and wild species was then calculated through the DICE coefficient (Dice, 1945) using
127 the Similarity for Qualitative data (SimQual) procedure of NTSyS-p.c. version 2.0 (Rohlf, 1998). The dendrogram was
128 constructed using the unweighted pair group method of arithmetic average (UPGMA) clustering and drawn with the NTSyS-p.c.
129 version 2.0 program (Rohlf, 1998).

130 Considering that pear accessions can be polyploid, the software SPAGeDi v.1.5 (Hardy and Vekemans, 2002) was used to
131 compute genetic information statistics, as this software supports analyses of datasets containing individuals with different ploidy
132 levels. Genetic statistics was performed on unique genotypes, excluding reference cultivars and duplicates and included number
133 of alleles per locus, effective number of allele, number of rare alleles per locus (alleles whose frequency in complex resulted
134 <0.05), number of genotype-specific alleles (unique alleles, present in only one accession), observed heterozygosity (Ho),
135 expected heterozygosity ($He=1-\Sigma pi^2$, where pi is the frequency of the ith allele) and Wright's fixation index (Fi). The probability
136 of identity (PI) was computed as defined by Paetkau et al. (1995), $PI= 2(\Sigma pi^2)^2 - \Sigma pi^4$, where pi is the frequency of the ith allele.

137 Then triploid accessions and duplicated genotypes were removed before performing the structure analysis using
138 STRUCTURE version 2.3.4 (Pritchard et al., 2000). For this analysis, another dataset was created, which included 454 unique
139 pear cultivars analysed by Fernández-Fernández (2010), as detailed in the publication by Ordidge et al., 2018 and 23 unique,
140 diploid Sardinian pear cultivars. To exclude further the duplicated genotypes from the analysis this dataset was previously
141 validated by repeating the NTSyS-p.c. cluster analysis. To investigate the structure using the genotype data of our dataset, a
142 Bayesian clustering method (Pritchard et al., 2000a; Falush et al., 2003) was applied using the STRUCTURE software. Previous
143 information was not used to define clusters. Independent runs were done by setting the number of clusters (k) from 2 to 10. Each
144 run included a burn-in period length set at 10,000 followed by 100,000 Markov Chain Monte Carlo (MCMC) number of repeats
145 under the admixture model. The most likely number of sub-clusters (k) was identified with STRUCTURE HARVESTER (Earl
146 and von Holdt, 2012) using the Δk described by Evanno et al. (2005). Genotypes were assigned to the group for which they
147 showed the highest membership coefficient, considering an accession strongly assigned to each partitioning level if its proportion
148 of ancestry (Q) was ≥ 0.80, otherwise they were considered as "admixed", according to Urrestarazu et al. (2012). The same
149 Structure parameters have been used for the Nested approach.

150

151 **3. Results**

152 As a result, the nine SSR markers employed showed a clear and easily readable peak amplification of the Sardinian 153 pears dataset composed of 82 local *P. communis* cultivars, 24 wild populations of *P. spinosa* and three international 154 reference cultivars (Table 1). Following the statistical analysis of the nine polymorphic loci, 101 unique genotypes were 155 detected within our dataset and 162 total alleles were found. The primer CH04e03 amplified a minimum of 12 different 156 alleles while a maximum of 30 alleles was found for the CH01f07a primer, as reported in Table 2. Furthermore, the average 157 number of alleles per locus found in the present research (Table 2) was notably different with respect to other large-scale 158 studies (9.4) on pear genetic diversity (Fernández-Fernández et al., 2006). The effective number of alleles varied between 159 1.66 (CH04e03) and 15.47 (CH01f07a), with an average of 8.45. The allele frequencies of the examined loci ranged between 160 0.002 and 0.775 (Supplementary Table 1) and for all studied SSR loci, it was possible to detect 26 specific rare alleles, nine 161 of which were found in the whole panel of analysed accessions for the SSRs EMPc11 and CH01f07a (Table 2). The 162 observed heterozygosity (Ho) ranged from 0.667 (CH04e03) to 1.00 (CH01d09 and EMPC11), with an average of 0.83 163 across loci, while the expected heterozygosity (He) ranged from 0.396 (CH04e03) to 0.935 (CH01f07a). Higher values of 164 Ho than He observed in eight loci out of nine resulted in negative Wright's fixation index (Fi) values, indicating a slight 165 excess of heterozygosity across these eight loci. For the remaining one locus (CH02b10), the F value was positive, meaning 166 a deficit of heterozygosity (Table 2). However, the average Fi (−0.101) was close to zero, suggesting that Sardinian pear 167 germplasm was comparable to an almost random mating unit. The highest probability of identity (PI) value (0.374) was at 168 CH04e03 locus and the lowest (0.009) at CH01f07a. The cumulative PI was 4.04×10^{-14}, indicating the possibility that two 169 randomly chosen individuals have the same SSR profile is not realistic.

170 Among accessions, 78 polyploids were identified (Table 3): 45 showed three alleles in more than one locus, of which 171 20 displayed three alleles in two loci, eight displayed three alleles in three loci, eight displayed three alleles in four loci, 172 eight displayed three alleles in five loci and two displayed three alleles in six loci. The remaining 33 accessions presented 173 only one locus with three alleles (Table 3).

174 The comparison of SSR profiles indicated four groups of diploid accessions with the same profile (Figure 1) allowing 175 to identify cases of possible synonymy such as 'Enosa' with 'E' Donna', as well as cases of misnomer such as 'Cozzon'e 176 Ainu' with 'Bragamotta', 'Alveghina' with 'Bau' and 'Reale' with 'William' (Fig. 1). Furthermore, the accessions 177 'Vacchesa' and 'Pira Ortine', 'Laconi 2' and 'Camusina Precoce', 'Bianca' and 'Mamoi' and 'Armungesa' and 'Mulargia' 178 showed very similar profile and have been discriminated for very small allelic differences at different loci (Supplementary 179 Table 2). In addition, the data set analysis showed that the *P. communis* group is phylogenetically separated from the *P.*

spinosa one, although some *P. communis* accessions, (e.g. 'Pira Cona Arrubia' and 'Pira di Urzulè'), revealed a closer genetic distance to *P. spinosa* than to *P. communis* (Fig. 1).

To better identify the presence of sub-clusters in this large dataset a structure analysis was performed (Fig. 3). Evanno's $\triangle k$ statistical analysis has shown unequivocally $k = 4$ ($\triangle k = 150$) as the most likely stratification level of the clusters (Fig. 2), which corresponded to the Sardinian pear gene pool (G1; yellow), the Japanese one (G2; blue), a group of late ripening cultivars (G3; green) and a group with the most famous standard cultivars (G4; red, Supplementary Table 3).

As far as *He,* the highest values were reached in G1 (0.834), followed by G3 having a value of 0.7069 while, G2 and G4 had similar values (0.6305 and 0.6308, respectively).

On behalf of the colour bar-plot it is possible to clearly evidence the four distinct gene groups (Fig. 3, Supplementary Table 3), supporting the $k = 4$ hypothesis. The fixation index (*Fst*) which, ranging from 0 to 1, could indicate no genetic divergence within the populations or complete extreme division respectively, was calculated for each gene group, and the values were very low ranging from 0.0015 (G1) to 0.3981 (G2) with intermediate values for G3 (0.1232) and G4 (0.2322).

In more detail, 105 accessions from G1 (including all Sardinian ones), 12 belonging to G2, 120 within G3 and 98 from G4 presented Q values higher than the 0.8 threshold value. The remaining 103 accessions had values lower than 0.8; therefore, they were considered as admixed (Supplementary Table 3). Within this large number of admixed accessions, 29 fit to G1, one to G2 while 43 and 30 were included in G3 and G4, respectively (Supplementary Table 3).

A restricted analysis of the 23 accessions belonging to Sardinian collections showed that the average of their Q values was 0.95 for G1, 0.01 for G2 (mainly composed of Japanese cultivars of *P. pyrifolia*) and 0.02 for both G3 and G4 (mainly composed of late cultivars of the centre of Europe and the classical pear cultivars used for breeding, respectively; Fig. 4).

In order to identify the presence of a possible gene flow between *P. spinosa* and *P. communis* a further structure analysis (nested approach) was conducted both on the cluster G1 dataset and on the panel of Sardinian accessions. The results of this analysis revealed that the two species shared the same gene pool and did not separate in different clusters (data not shown).

4. Discussion

Sardinia possesses a great plant diversity with numerous local cultivars and wild pear that are likely differentiated according to soil and climatic variable conditions as well as by anthropic selection (Agabbio et al., 1986, 2015). Indeed, local cultivars own a high variability for several agronomic traits such as fruit size, ripening time, soil adaptability and resilience to environmental conditions, characterised by high temperatures and scarce water availability during the summer period (from June to September). Modern breeding programs have never exploited this genetic diversity. In this work, relationships among the local, old, safeguarded cultivars and wild related species were inferred by SSR marker analysis.

211 Reproducibility and coherence in amplified peak readings in the references 'William', 'Kaiser' and 'Abate' have
212 allowed us to make sure that the SSRs used in this work are valuable markers to define pear germplasm diversity.
213 The identification of one synonymy ('Enosa' with 'È Donna') in the Sardinian germplasm emphasised the importance
214 of linking referee collections with powerful tools such as molecular markers aimed at avoiding repetitions and promoted
215 the withdrawal of duplicates.
216 The use of reliable SSRs is essential for the effective differentiation of cultivars and the establishment of genetic
217 relationships such as homonymies and synonymies. Three putative misnomers are between 'Reale' and 'William',
218 'Alveghina' and 'Bau' and 'Bragamotta' and 'Cozzonn'e Ainu' that are known to be different cultivars from their
219 descriptors.
220 A high similarity was found between the Sardinian cultivars 'San Domenico' and 'Beurré Giffard' an old French
221 summer pear that is usually picked at the end of July or at the beginning of August. 'San Domenico' is also an early-
222 ripening pear starting from June up to mid-July, with a buttery melting flesh similar to 'Beurré Hardy'.
223 The overall diversity analysis proved the allelic interchange between accessions of *P. communis* and *P. spinosa* or vice
224 versa. The alignment of the two datasets confirmed the phylogenetical rift of *P. spinosa*, and on behalf of the allelic
225 frequency analysis, highlighted the magnitude of shared alleles. After dataset analysis, ''De Puleu', 'Buttidu de Austu' and
226 'Mela' resulted in four alleles in a single locus. In the present work, the number of alleles in each locus and the range of
227 achieved values diverge from the data available in the literature (Table 2). The overall allelic diversity evidenced by
228 employing nine SSRs revealed a high polymorphism in the analysed Sardinian germplasm.
229 The phylogenetic proximity between the accessions 'Pira di Urzulè' and 'Pira Cona Arrubia' with the wild populations
230 of *P. spinosa* can be explained by the fact that these cultivars, according also to morphological and chemical similarities,
231 may be due to of hybridisation between *Pyrus* species (*P. spinosa* and *P. communis*) as reported in other geographical
232 regions rich in the genus *Pyrus*. A botanical hybrid between *P. communis* and *P. spinosa* = *P.* × *jordanovii* has been
233 discovered in Pirin Mountains of Bulgaria (Dostálek, 1984) and other hybridisations between these two species have been
234 already described (Vincent et al., 2013). This phenomenon was also recently evidenced in the pear germplasm of the Mount
235 Etna in which an allelic interchange was found between *P. communis*, *P. pyraster* and *P. spinosa* (Bennici et al., 2018).
236 The high number of putative triploids in Sardinian cultivars can be explained by the fact that anthropic selection pressure
237 has sorted out the triploid accessions because of the generally larger size of the fruit. It is known that, generally, the triploid
238 apple and pear accessions bear larger fruits (Ashton and Spigel-Roy, 1985). This phenomenon could explain the relatively
239 high percentage of triploids among the pear old varieties and landraces. A percentage of 15% of triploids was recently
240 described in the pear local germplasm of Friuli Venezia Giulia, a region in the North-eastern of Italy (Baccichet et al., 2020)
241 while the very high percentage of 24.2% was described in a panel of German and Romanian accessions (Puskas et al.,

242 2015). Analogously to pear, a high number of triploid accessions (about 10% on more than 2000 analysed samples) was
243 also found to be present in apple landraces and local germplasms at European level (Urrestarazu et al., 2016). A detailed
244 study in *Pyrus* species revealed that all species are diploid, with 2n = 34, although different cultivars and variants are triploid
245 (Zielinski et al. 1967), so, in several cases, the marker might have amplified another locus of the homologous genome
246 (Pierantoni et al. 2004).

247 Thanks to the existence of a large dataset created by Fernández-Fernández et al. (2006), which covers *Pyrus*'s
248 international and local cultivars, comparing these cultivars with our dataset has been possible, thus increasing the
249 availability of useful data for future analysis. In fact, the use of the same markers in both analyses made it possible to align
250 the two datasets.

251 The SSR alleles used in the structure analysis (Fig 2) allowed us to identify four groups. The Sardinian accessions
252 belong to G1, together with a large group of the Italian cultivars that have been analysed in the Fernández-Fernández dataset
253 (Fernández-Fernández et al., 2006, Sehic et al., 2010) and French early ripening cultivars. These results agree with the
254 observations of Bennici et al. (2018) that showed a notable contribution to the genetic makeup of many Italian cultivars,
255 especially ancient ones. The relationship found among pear genotypes from Sardinia, Italy and France may have historical
256 origin by vegetative propagation of plant material exchanged between Italy and France mainly during the Sardinian
257 Piedmont reign (1720-1861; Agabbio et al., 2015).

258 The Q values of *P. communis* and *P. spinosa* accessions are very close as confirmation of an allelic interchange between
259 the wild and the cultivated genotypes, to support the idea that no significant gene flow exists between the Sardinian gene
260 pool G1 and the ones of G2, G3 and G4 (Fig. 4).

261 The very low *Fst,* observed in all four groups indicates that genetic variability is not massively linked to the structure
262 of the population analysed. In fact, although a difference exists between the statistically valid groups, we are in a fairly
263 homogeneous gene pool.

264

265 **5. Conclusion**

266 This document describes, for the first time, the genetic diversity of domesticated and wild Sardinian pears by considering
267 a sample of over 100 accessions, to characterise the cultivars' genetic structure and studying the influence of wild species
268 on specific local germplasm.. The development of a large dataset allowed us to use reliable classification criteria and
269 estimate genetic distances among cultivars. The availability of a large pear dataset in literature allows the identification or
270 confirmation of homonymies and synonymies that could occur in different germplasm accessions. This dataset provides an
271 overview of the history of diversification of the pear germplasm in Sardinia, diversification that was steered by man and

272 influenced by the high environmental and climatic variability present on the island. A clear allelic interchange between *P.*
273 *communis* and *P. spinosa* accessions was detected. This Sardinian germplasm represents a very important source of genetic
274 diversity that was never investigated at the molecular level and that could be exploited in new breeding programs aimed at
275 improving the resilience of the new pear cultivars in a new context characterised by deep climatic changes.

276

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283

284 Figure and table captions

285 **Table 1**. In uppercase cultivars of *P. communis* (*P.c*) and (*P.c**) and in lowercase accessions of *P. spinosa* (*P.s*) analysed
286 in this study. In bold, reference cultivars.

287 **Table 2.** Characteristics of the nine SSRs studied in Sardinian *P. communis* and *P. spinosa* accessions.

288 **Table 3.** Triploids and tetraploids set found in the Sardinian *P. communis* cultivars (in uppercase) and in *P. spinosa* wild
289 populations (in lowercase).

290 **Fig. 1.** Dendrogram of 109 pear cultivars from the Sardinian germplasm based on DICE coefficient calculated from the
291 allele frequencies of 194 alleles found in nine SSR loci. The *P. communis* is in uppercase letters, while the *P. spinosa*
292 wild population is in lowercase. In the black circle the misnomer found in the Sardinian *Pyrus* accessions. In the black
293 rectangles are the cultivars that are differentiated for less than 10 alleles. In bold are the reference cultivars.

294 **Fig. 2.** Slope rate change estimates of the log probability curve ($\triangle$K) calculated according to Evanno
295 et al. (2005) plotted against number of cluster (K).

296 **Fig. 3.** Clusters identified in the first round structure analysis: Sardinian (yellow), Japanese (blue); late cultivars (green);
297 the most famous standard cultivars (red).

298 **Fig. 4.** Genetic composition of the Sardinian gene pool for K=4 groups inferred with Structure and described by the mean
299 Q value distribution. Sardinian (G1); Japanese (G2); late ripening (G3); the most famous standard cultivars (G4).

300 Supplementary Material

301 **Supplementary Table 1:** Allele frequency of nine SSRs in Sardinian *Pyrus* germplasm. Rare alleles are indicated in bold
302 and unique alleles are evidenced by underling.

303 **Supplementary Table 2:** Sardinian pear cultivars with similar genotypes. In bold, allele that differentiates the genotype.

304 **Supplementary Table 3:** Classification of 454 genotypes including Sardinian *Pyrus* germplasm and pear cultivars
305 analysed by Fernández-Fernández et al. (2006) by structure using 9 SSR loci in K = 4 reconstructed populations.

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