Genetic Diversity of Stone Fruit Cultivars Preserved On-Farm in Southern Spain

P. Rallo¹*, M. Rocío Jiménez¹, L. Casanova¹, A. Morales-Sillero¹, and M. Paz Suárez¹

ABSTRACT

Old traditional cultivars are valuable genetic resources for crop improvement, but a great number of them have disappeared in the past century. This study aimed to characterize traditional cultivars of different Prunus species collected in small family orchards in southwestern Spain and to evaluate their genetic diversity and relationships. One hundred and twelve accessions belonging to 36 traditional cultivar denominations were analyzed using eight SSR loci transferable across the genus Prunus. The most useful loci to analyze different Prunus species were UDP96-005, BPPCT-002, UDP98-410 and ps02a12. A total of 152 alleles were observed, and 112 were unique to certain species. Sixty-eight different genotypes were found, revealing the possible existence of homonyms among traditional cultivar names. The clustering analysis was consistent with the taxonomic classification of the different species studied and with the geographical origins of the accessions within each species. The results showed wide genetic variability of traditional cultivars of stone fruits grown in small family orchards, which highlights the need to preserve them using both in-situ and ex-situ strategies. Twenty-eight of these accessions are currently conserved ex-situ at the University of Sevilla, Spain. The use of highly transferable SSRs has been proven as efficient in multi-species surveys performed on-farm.

Keywords: Genetic resources, Homonyms Local varieties, Microsatellites, Prunus.

INTRODUCTION

Agricultural biodiversity conservation has become a global priority to ensure the nutrition of future generations in a changing world and thus has been confirmed by the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2001). Local varieties kept and cultivated by farmers are an important part of plant genetic resources that need to be protected since many of them are currently at serious risk of being lost (Esquinas-Alcázar, 2005).

The genus Prunus accounts for more than 200 species (Rehder, 1940), including some of the most important fruit crops, namely, peach (Prunus persica (L.) Batsch), European plum (Prunus domestica L.), Japanese plum (Prunus salicina Lindl), apricot (Prunus armeniaca L.) and sweet cherry (Prunus avium L.). Stone fruits have been traditionally grown in southern Spain, but since the late 1970s, most of the local cultivars have been replaced by highly productive varieties from international breeding programs. At present, many traditional cultivars have disappeared forever (Martin et al., 2011), but fortunately, in some areas, they are still grown in small family orchards maintained by elderly farmers. A regional germplasm survey was conducted in Andalusia (Spain) by our group to locate old cultivars from different fruit species (Rallo et al., 2011; Perez-Romero et al., 2015).

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A fast and reliable method for initial cultivar discrimination is essential in order to manage these on-farm surveys since homonyms, synonyms, and the use of local names are very common in traditional plant material (Garcia-Munoz et al., 2012; Gouta et al., 2012; Trujillo et al., 2014). DNA markers, particularly simple sequence repeats (SSRs), have become an invaluable tool in germplasm characterization. Many SSRs have been developed for different Prunus species: peach (Cipriani et al., 1999; Testolin et al., 2000; Dirlewanger et al., 2002), apricot (Vilanova et al., 2006), cherry (Vaughan and Russell, 2004), Japanese plum (Mnejja et al., 2004) and almond (Mnejja et al., 2005). The transferability of SSRs to other Prunus species is of particular interest for genotyping traditional tree cultivars preserved on-farm since a mixture of species is usually cultivated in the same orchard.

This study aimed to characterize 112 accessions of stone fruit cultivars collected from small farms in southwestern Spain and to evaluate their genetic diversity and relationships. Additionally, we aimed to explore the usefulness of eight cross-transferable SSR loci for multi-species characterization, which is important in plant surveys performed on-farm.

**MATERIALS AND METHODS**

**Plant Material and DNA Isolation**

A total of 112 trees belonging to 36 traditional cultivar denominations of apricot, sweet cherry, peach, European plum and other plums were included in this study (Table 1). The trees were located in 16 different orchards in four municipal districts (Galaroza, Navahermosa, Olivares and Constantina) in the provinces of Seville and Huelva (South West Spain) (Figure 1). When possible, more than one tree under the same cultivar name was sampled, especially if they were grown in different orchards.

Young leaf samples were collected from each tree, and DNA was isolated according to De La Rosa et al. (2002). The amount and quality of DNA was estimated visually with lambda DNA marker (Promega Biotech Iberica, Madrid, Spain) on 1% agarose gel stained with ethidium bromide.

**PCR Amplification and Detection**

Eight SSR loci previously proven to be transferable to different Prunus spp. (Wünsch, 2009) were used in this study: ps12a02 (Downey and Iezzoni, 2000); UDP96-005 and UDP98-409 (Cipriani et al., 1999); UDP98-410 (Testolin et al., 2000); BPPCT-002, BPPCT-004, BPPCT-010, and BPPCT-026 (Dirlewanger et al., 2002) (Supplementary Table A).

Forward primers were labeled with fluorescent dyes 6-FAM, HEX, Atto 550 or Atto 565 (Biomers.net, Ulm, Germany). PCR reactions were performed in a final volume of 20 μL containing 20 ng genomic DNA, 1X Buffer [75 mM Tris HCl, pH 9, 50 mM KCl, 20 mM (NH₄)₂SO₄], 0.20 mM dNTPs, 4.00 mM MgCl₂, 0.20 μM of each primer, and 0.07 U μL⁻¹ of Taq polymerase (Biotools, Madrid, Spain). Amplification was carried out on a thermal cycler (GeneAmp® PCR System 2700, Applied Biosystems®, Foster City, CA, USA) under the following conditions: an initial step of 2 minutes at 94°C, 35 cycles of 45 seconds at 94°C, 45 seconds at 57°C and 1 minute at 72°C, and a final step of 5 minutes at 72°C.

The amplified products were detected by capillary electrophoresis in an ABI 3130XL system (Applied Biosystems®, Foster City, CA, USA) using the standard GeneScan-500 LIZ (Applied Biosystems®, Foster City, CA, USA). The electrophoresis results were analyzed using the GeneScan v 3.7 software.

**Data Analysis**

The sizes of all alleles detected were determined at the eight loci analyzed. Samples were scored for the presence (1) or absence (0) of each allele detected per locus.
Genetic similarity according to the Nei and Li (1979) coefficient was estimated among all the Prunus accessions. Cluster analysis and construction of a dendrogram were performed with the Unweighted Pair-Group Method (UPGMA) using Arithmetic averages. The NTSYS-pc v.2.02 package (Exeter software, Setauket, NY, USA) was used for all calculations. BPPCT-026 was not considered for cluster analysis due to the large number of failures.

To assess the level of polymorphism and genetic information of the SSR loci employed, the following parameters were calculated for the whole sample (Table 2) and for each species (Table 3): number of alleles per locus, number of unique alleles per species, observed Heterozygosity (Ho, direct count of heterozygous individuals over total number of genotypes), expected Heterozygosity according to Nei (1973) (He= 1-∑pi^2, where pi is the frequency of the ith allele) and the
Table 1. Cultivar accessions of various *Prunus* species analyzed in this study.

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivar, accession</th>
<th>Municipal district (Province)</th>
<th>Orchard name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricot</td>
<td>Isidro 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>La Era 2</td>
</tr>
<tr>
<td></td>
<td>Isidro 3, 4</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Cachón</td>
</tr>
<tr>
<td></td>
<td>De Parma 1, 2, 3</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Macario 1</td>
</tr>
<tr>
<td><em>Prunus armeniaca</em> L.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cherry</td>
<td>Aranjuez 1, 2</td>
<td>Galaroza (Huelva)</td>
<td>Los Roblecillos</td>
</tr>
<tr>
<td></td>
<td>Burral 1</td>
<td>Galaroza (Huelva)</td>
<td>La Confesa 1</td>
</tr>
<tr>
<td></td>
<td>Burral 2</td>
<td>Galaroza (Huelva)</td>
<td>La Confesa 2</td>
</tr>
<tr>
<td></td>
<td>Burral 3, 4</td>
<td>Galaroza (Huelva)</td>
<td>Los Roblecillos</td>
</tr>
<tr>
<td></td>
<td>Blandillo 1, 2, 3, 4, 5, 6, 7, 8, 9</td>
<td>Constantina (Sevilla)</td>
<td>San Gabriel</td>
</tr>
<tr>
<td></td>
<td>Negro 1, 2, 3, 4</td>
<td>Constantina (Sevilla)</td>
<td>San Gabriel</td>
</tr>
<tr>
<td></td>
<td>Tierno 1</td>
<td>Navahermosa (Huelva)</td>
<td>Unnamed</td>
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<tr>
<td></td>
<td>Tierno 2</td>
<td>Galaroza (Huelva)</td>
<td>Los Roblecillos</td>
</tr>
<tr>
<td>Peach</td>
<td>Almagreño</td>
<td>Galaroza (Huelva)</td>
<td>Los Roblecillos</td>
</tr>
<tr>
<td><em>Prunus persica</em> L.</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Almagreño amarillo sin picón 1, 2, 3, 4</td>
<td>Galaroza (Huelva)</td>
<td>La Confesa 1</td>
</tr>
<tr>
<td></td>
<td>Almagreño blanco 1</td>
<td>Galaroza (Huelva)</td>
<td>La Confesa 1</td>
</tr>
<tr>
<td></td>
<td>Almagreño blanco 2</td>
<td>Galaroza (Huelva)</td>
<td>Huerta Venecia</td>
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<td></td>
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<td>Olivares (Sevilla)</td>
<td>Huerta pelaquero</td>
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<td>Paraguayo 2</td>
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<td>Prisco 1, 2</td>
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<td>Tardío o menudillo 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Macario 2</td>
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<td></td>
<td>Tardío o menudillo 2</td>
<td>Olivares (Sevilla)</td>
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<tr>
<td>European Plums</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><em>Prunus domestica</em> L.</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Almizqueña 1, 2, 3</td>
<td>Olivares (Sevilla)</td>
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<td>Huerta Macario 1</td>
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<td>Blanquilla blanca 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>La Confesa 1</td>
</tr>
<tr>
<td></td>
<td>Blanquilla blanca 3</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Cachón</td>
</tr>
<tr>
<td></td>
<td>Blanquilla coloradas 1</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Venecia</td>
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<tr>
<td></td>
<td>Blanquilla coloradas 2</td>
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<td>Huerta Cachón</td>
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<td>Bomba larga 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Macario 1</td>
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<td></td>
<td>Bonitas monjas 1</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Venecia</td>
</tr>
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<td></td>
<td>Bonitas monjas 2, 3</td>
<td>Olivares (Sevilla)</td>
<td>Los Rubiales</td>
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<td>Botella 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>La Era 1</td>
</tr>
<tr>
<td></td>
<td>Botella 3</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Macario 1</td>
</tr>
<tr>
<td></td>
<td>Botella 4</td>
<td>Olivares (Sevilla)</td>
<td>Los Rubiales</td>
</tr>
<tr>
<td></td>
<td>Claudia antigua 1</td>
<td>Olivares (Sevilla)</td>
<td>La Era 2</td>
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<td>Claudia antigua 2, 3</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Macario 1</td>
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<tr>
<td></td>
<td>Confitero 1, 2, 3, 4</td>
<td>Galaroza (Huelva)</td>
<td>Francisco &quot;colorao&quot;</td>
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<td></td>
<td>De uña 1, 2, 3, 4, 5</td>
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<td>Huerta Venecia</td>
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<td>El Injertal</td>
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<td>Olivares (Sevilla)</td>
<td>Huerta Cachón</td>
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<tr>
<td></td>
<td>Oloroso 1</td>
<td>Galaroza (Huelva)</td>
<td>Huerta Venecia</td>
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<td>San Gabriel claudia 1, 2</td>
<td>Constantina (Sevilla)</td>
<td>San Gabriel</td>
</tr>
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<td>San Gabriel negra 1, 2</td>
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<td>San Gabriel</td>
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<tr>
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<td>Sanjuaniega 1, 2, 3, 4</td>
<td>Galaroza (Huelva)</td>
<td>La Confesa 1</td>
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<tr>
<td></td>
<td>Sanjuaniega 5</td>
<td>Galaroza (Huelva)</td>
<td>Los Roblecillos</td>
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<tr>
<td>Other plums</td>
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<td>Bomba redonda 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Macario 1</td>
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<td></td>
<td>Ciruelo guindo 1, 2</td>
<td>Olivares (Sevilla)</td>
<td>Huerta Cachón</td>
</tr>
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<td>Hartabruco 1, 2</td>
<td>Galaroza (Huelva)</td>
<td>Los Roblecillos</td>
</tr>
<tr>
<td></td>
<td>Oloroso 2</td>
<td>Galaroza (Huelva)</td>
<td>Huerta Venecia</td>
</tr>
</tbody>
</table>
Table 2. Summary of SSR genetic diversity parameters calculated for 112 accessions of different stone fruit species: locus name, number of genotypes detected, size range of the amplified fragments, number of alleles, number of unique alleles per species, observed Heterozygosity (Ho) and Power of Discrimination (PD).

<table>
<thead>
<tr>
<th>SSR locus</th>
<th>Number of genotypes</th>
<th>Range size (bp)</th>
<th>Number of alleles</th>
<th>Number of unique alleles/spp.</th>
<th>Ho</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ps02a12</td>
<td>36</td>
<td>(107-194)</td>
<td>21</td>
<td>14</td>
<td>0.89</td>
<td>0.96</td>
</tr>
<tr>
<td>UDP98-410</td>
<td>28</td>
<td>(112-144)</td>
<td>19</td>
<td>13</td>
<td>0.71</td>
<td>0.95</td>
</tr>
<tr>
<td>UDP98-409</td>
<td>23</td>
<td>(114-164)</td>
<td>18</td>
<td>14</td>
<td>0.77</td>
<td>0.92</td>
</tr>
<tr>
<td>UDP96-005</td>
<td>33</td>
<td>(101-174)</td>
<td>25</td>
<td>22</td>
<td>0.75</td>
<td>0.95</td>
</tr>
<tr>
<td>BPPCT-002</td>
<td>40</td>
<td>(165-232)</td>
<td>22</td>
<td>14</td>
<td>0.69</td>
<td>0.95</td>
</tr>
<tr>
<td>BPPCT-004</td>
<td>26</td>
<td>(163-214)</td>
<td>19</td>
<td>17</td>
<td>0.74</td>
<td>0.92</td>
</tr>
<tr>
<td>BPPCT-010</td>
<td>21</td>
<td>(112-155)</td>
<td>11</td>
<td>8</td>
<td>0.60</td>
<td>0.91</td>
</tr>
<tr>
<td>BPPCT-026</td>
<td>22</td>
<td>(121-186)</td>
<td>17</td>
<td>10</td>
<td>0.63</td>
<td>0.92</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td></td>
<td>152</td>
<td>112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-</td>
<td></td>
<td>19</td>
<td>14</td>
<td>0.72</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Power of Discrimination (PD = $1 - \Sigma g_i^2$, where $g_i$ is the frequency of the $i$th genotype (Kloosterman et al., 1993)). Plum samples were excluded from Ho calculations due to their polyploid nature.

RESULTS AND DISCUSSION

Cross-Transferable SSR Amplification and Polymorphism

The eight SSR loci used were selected among a set of 13 microsatellites previously proven to be polymorphic and transferable across different Prunus species (Wünsch, 2009). Although amplification products were obtained from all loci (Tables 2 and 3), there were differences in the number of amplified accessions among them. Particular failures were related to the species studied, indicating a possible problem of transferability: 10 out of the 19 peach samples failed to amplify ps12a02, the only SSR originating from cherry and, similarly, no amplification was obtained for BPPCT-026, developed for peach, in 19 of the 21 cherry samples (data not shown). Both species (P. persica and P. avium) belong to two of the most distant Prunus subgenera: Amygdalus and Cerasus, respectively (Lee and Wen 2001). Indeed, Mnejja et al. (2010) found in Prunus an inverse relationship between genetic distance and transferability.

Multiple amplification (three to six amplicons) was recorded in 28 plum accessions presumably belonging to P. domestica, a hexaploid species. These results confirm the polyploid nature and the abundance of European plums found in the survey. Other authors reported three to six amplicons per individual in samples of the same species (Bouhadida et al., 2009; Wünsch, 2009; Gharbi et al., 2014; Kazija et al., 2014; Sehic et al., 2015; Makovics-Zsohar et al. 2017).

Considering all accessions (Table 2), a total of 152 alleles were obtained (112 unique to certain species), ranging from 11 (8 unique) alleles for locus BPPCT-010 to 25 (22 unique) for UDP96-005. The values of observed Heterozygosity (Ho) varied between 0.60 for BPPCT-010 and 0.89 for ps12a02. Few differences were found for the Power of Discrimination (PD), which had values ranging from 0.91 to 0.96. The Ho and PD values were very similar to those obtained by Wünsch (2009), who analyzed 27 genotypes of ten Prunus species.
Table 3. SSR genetic diversity parameters calculated for each *Prunus* species analyzed: *locus* name, number of genotypes, size range of the amplified fragments, number of alleles, observed (Ho) and expected (He) Heterozygosities and Power of Discrimination (PD).

<table>
<thead>
<tr>
<th>Species</th>
<th>SSR locus</th>
<th>Number of genotypes</th>
<th>Range size (bp)</th>
<th>Number of alleles</th>
<th>Ho</th>
<th>He</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apricot</td>
<td>ps02a12</td>
<td>2</td>
<td>154-184</td>
<td>3</td>
<td>1</td>
<td>0.62</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>UDP98-410</td>
<td>2</td>
<td>112-118</td>
<td>2</td>
<td>0.43</td>
<td>0.34</td>
<td>0.49</td>
</tr>
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<td></td>
<td>UDP98-409</td>
<td>2</td>
<td>139-164</td>
<td>3</td>
<td>0.57</td>
<td>0.65</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>UDP96-005</td>
<td>2</td>
<td>109-144</td>
<td>4</td>
<td>1.00</td>
<td>0.75</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>BPPCT-002</td>
<td>2</td>
<td>187-191</td>
<td>3</td>
<td>1</td>
<td>0.65</td>
<td>0.49</td>
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<tr>
<td></td>
<td>BPPCT-004</td>
<td>1</td>
<td>196</td>
<td>1</td>
<td>4.00</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>BPPCT-010</td>
<td>2</td>
<td>120-124</td>
<td>2</td>
<td>0.57</td>
<td>0.41</td>
<td>0.49</td>
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<tr>
<td></td>
<td>BPPCT-026</td>
<td>1</td>
<td>220</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Mean

|                   | 2.5 | 0.70 | 0.49 | 0.37 |

| Cherry | ps02a12 | 12   | 158-182 | 8    | 0.62 | 0.67 | 0.82 |
|        | UDP98-410 | 5    | 120-128 | 4    | 0.91 | 0.66 | 0.69 |
|        | UDP98-409 | 3    | 114-125 | 3    | 0.86 | 0.57 | 0.57 |
|        | UDP96-005 | 5    | 119-139 | 4    | 0.38 | 0.54 | 0.68 |
|        | BPPCT-002 | 6    | 177-183 | 4    | 0.67 | 0.57 | 0.71 |
|        | BPPCT-004 | 2    | 178-194 | 3    | 1    | 0.55 | 0.19 |
|        | BPPCT-010 | 2    | 118-120 | 2    | 0.14 | 0.13 | 0.24 |
|        | BPPCT-026 | 2    | 123-186 | 4    | 0.5  | 0.72 | 0.67 |

Mean

|                   | 4    | 0.64 | 0.55 | 0.57 |

| Peach | ps02a12 | 5    | 107-166 | 6    | 0.67 | 0.81 | 0.77 |
|       | UDP98-410 | 5    | 140-144 | 4    | 0.32 | 0.57 | 0.71 |
|       | UDP98-409 | 2    | 128-130 | 2    | 0.11 | 0.10 | 0.18 |
|       | UDP96-005 | 9    | 157-174 | 7    | 0.28 | 0.81 | 0.86 |
|       | BPPCT-002 | 2    | 229-232 | 2    | 0    | 0.21 | 0.21 |
|       | BPPCT-004 | 2    | 198-200 | 2    | 0    | 0.48 | 0.49 |
|       | BPPCT-010 | 1    | 132     | 1    | 0    | 0    | 0.0 |
|       | BPPCT-026 | 7    | 133-146 | 6    | 0.05 | 0.77 | 0.81 |

Mean

|                   | 0.64 | 0.55 | 0.72 | 0.81 |

| Plum* | ps02a12 | 17   | 146-194 | 14   | 1    | 0.92 |
|       | UDP98-410 | 16   | 114-142 | 15   | 0.79 | 0.93 |
|       | UDP98-409 | 16   | 123-158 | 14   | 0.95 | 0.88 |
|       | UDP96-005 | 17   | 101-155 | 13   | 0.98 | 0.91 |
|       | BPPCT-002 | 30   | 177-232 | 21   | 0.89 | 0.94 |
|       | BPPCT-004 | 31   | 163-214 | 15   | 0.96 | 0.91 |
|       | BPPCT-010 | 16   | 120-155 | 9    | 0.94 | 0.9  |
|       | BPPCT-026 | 12   | 121-170 | 13   | 0.91 | 0.88 |

Mean

|                   | 3.75 | 0.18 | 0.47 | 0.50 |

When comparing the results within each species (Table 3), greater differences among SSRs were found. For apricot, BPPCT-004 and BPPCT-026 were monomorphic. The low number of apricot genotypes (only two) may explain the lack of polymorphism since most authors reported polymorphic results for those SSRs (Wünsch, 2009; Bourguiba et al., 2010; Lamia et al., 2010). Within the cherry species, ps12a02 was clearly the most informative locus with the highest PD (0.82), number of genotypes, and alleles. For the peach group, general levels of diversity (Ho= 0.18, He= 0.47 and PD= 0.50) were lower than those observed for the other species. Most of the samples were homozygous as revealed by the low Ho, below 0.32 in most loci. BPPCT-010 was monomorphic, although this locus was
Genetic Diversity of Stone Fruit Cultivars

reported to be polymorphic for peach by other authors (Dirlewanger et al., 2002; Wünsch, 2009; Font et al., 2013). The lower levels of variability in peach could be attributed to its self-compatibility (Hegedus et al., 2006) along with similar origins and shared pedigree (Martínez-Gómez et al., 2003). Unlike the rest of the species, BPPCT-026 was one of the most polymorphic loci in peach (Table 3). All eight SSRs were very polymorphic in plums, the largest group of samples (65 accessions). The diversity parameters were very high among plums: mean Ho= 0.93 and PD = 0.91. BPPCT-002 identified the largest number of genotypes in plum (30).

Genetic Variability of Traditional Cultivars

The variability detected within the species in this work was high (Table 3) despite the restricted geographical area of the survey. Large genetic and morphological variability is commonly found in local germplasm as reported in various Prunus species in other countries (Gouta et al., 2010, 2012; El Hamzaoui et al., 2013; Rakonjac et al., 2014; Öz et al., 2013; Sehic et al., 2015). This wide variability brings to light the richness of traditional cultivars.

The results obtained in this work are comparable to other studies on the Spanish germplasm of stone fruits. Particularly in peach, Wünsch et al. (2006) analyzed a set of 85 local Spanish cultivars and found similar average number of alleles per locus (3.5). Bouhadida et al. (2011) and Font et al. (2013) found a greater mean number of alleles per locus (6.73 and 5.10), Ho (0.23 and 0.48), He (0.57 and 0.49) and PD (0.66 and 0.47) among 62 and 43 local Spanish cultivars, respectively. The larger number of cultivars along with the higher polymorphism levels of the SSRs used by these authors may be behind these differences. In our case, SSR loci were chosen on the basis of their transferability to other Prunus species rather than for their level of polymorphism. In cherry, Wünsch and Hormaza (2004) analyzed 28 local cultivars from western Spain. Their results for the three common loci used (ps12a12, UDP 96-005 and UDP 98-409) revealed similar numbers of alleles/loci and similar size ranges to ours. Only two apricot cultivars (‘Isidro’ and ‘De Parma’) were analyzed in our work, but they were clearly distinguishable with each of the six SSRs that were polymorphic for the species. The similarity coefficient between both cultivars was lower than those reported by Martin et al. (2011) out of 34 old apricot varieties.

There are very few references of the evaluation of Spanish local plums with SSRs (Laquidain et al., 2011; Gharbi et al., 2014), and they mostly refer to a limited number of accessions of ‘Reine Claude Verte’. A larger number of traditional European plums from different countries (Öz et al., 2013; Kazija et al., 2014; Sehic et al., 2015; Makovics-Zohar et al., 2017) have been recently studied with SSRs and, as in this work, high genetic diversity has been found, with similar number of alleles and size ranges in the case of the only common loci used, UDP 96-005.

Genetic Relationships among Accessions

The UPGMA dendrogram obtained (Figure 2) clearly separates the accessions in five clusters according to the botanical species: apricot (P. armeniaca), plum 1 (polyploid plum samples presumably belonging to P. domestica), peach (P. persica), plum 2 (diploid plum samples) and cherry (P. avium). Relationships among these groups are partly consistent with the taxonomic classification of stone fruit species (Rehder, 1940). Peach belongs to the subgenus Amygdalus, apricot and plums belong to the subgenus Prunus or (Prunophora) and sweet cherry belongs to the subgenus Cerasus, which is the most distant group. In our work, the apricot cluster and the polyploid plum cluster (Plum 1) grouped together, consistent with the closer relationship between apricots and
Figure 2. Dendrogram of 112 accessions of local stone fruit cultivars based on UPGMA analysis of seven SSR loci (BPPCT-026 not included). Symbols refer to the geographic locations of the collected accessions [Constantina (▲), Galaroza (□), Navahermosa (■), Olivares (●)].
European plums as members of the *Prunus* subgenus. The peach cluster was also closer to plums and apricots than to cherry, as *Amygdalus* and *Prunus* subgenera are closer to each other than either is to the *Cerasus* subgenus. Similar results with a wider number of species per subgenus have been reported (Lee and Wen, 2001; Bortiri et al., 2006, Bouhadida et al., 2007). In contrast, in this work, diploid plums were placed in a different cluster (Plum 2) closer to cherry than to the other plum cluster (Plum 1). Plum 2 comprises six genotypes with very low similarity coefficients among them. Some of these accessions may be seedlings or wild plums initially used as rootstocks but presumably grown as non-grafted trees after scion loss. The names of some of them, ‘Hartabruto’ (meaning “very rustic”) or ‘Ciruelo guindo’ (meaning “plums with the appearance of sour cherries”) seem to confirm the possibility that they could derive from rootstocks and be taxonomically closer to the *Cerasus* subgenus.

Samples from each municipal district tended to group together; this was especially remarkable within plum 1 and cherry clusters. Certain accessions were clustered according to their geographical origin as other authors found (Bouhadida et al., 2011).

**Traditional Cultivar Fingerprinting**

Sixty-eight unique genetic profiles were found for 36 cultivar names (Table 2; Figure 2), which highlight the occurrence of homonyms in the collected sample, i.e., using the same name for different genotypes. This is very common in traditional cultivars of different fruit species: apple (Halasz et al., 2011; Pina et al., 2014), almond (Gouta et al., 2012), European plums (Makovics-Zsohar et al., 2017), grapes (Buhner et al., 2010; Garcia-Muñoz et al., 2012) and olive (Trujillo et al., 2014), since most of the names refer to generic morphological traits, leading to errors and mis-named cultivars. For example, in the present work, many names (Table 1) refer to fruit color (‘Almagreño blanco’, ‘Almagreño amarillo’, ‘Blanquilla blanca’, ‘Blanquilla colorada’, ‘Negro’), flesh texture (‘Blandillo’, ‘Tierno’, both meaning “soft”), fruit shape (‘Bomba larga’, ‘Bomba redonda’, ‘Botella’, ‘Hermosas’, meaning “long or rounded bomb-shaped”, “bottle” and “beauties”, respectively) or other fruit traits (‘Confitero’, ‘Oloroso’, ‘Tardío’, meaning “for sweet making”, “fragrant” and “late ripe”, respectively). Homonyms were found in all species but apricot. In some cases, slight differences were found among accessions with the same name, such as ‘Gruño’, ‘Confitero’, ‘Frailes’ or ‘Blanquilla blanca’ plums (Figure 2), indicating the possible existence of spontaneous mutations in grafted trees as a source of clonal variation. Hybridization events may also be behind the larger variations observed among other accessions such as in ‘Blandillo’ cherry samples. The use of seedlings for plant propagation has been reported as a common practice by farmers in different *Prunus* species (Martín et al., 2011; El Hamzaoui et al., 2013). In other cases, as for ‘Oloroso’ accessions, differences were so big that mislabeling may have occurred (cluster Plum 1 and Plum 2).

In the 1950s, an extensive survey and inventory mission for different fruit species were carried out in Spain (Herrero, 1964). The survey included some of the municipal districts that have been explored in this work (Figures 1 and 2). Olivares was described as a location with a long tradition of cultivating different fruit species, with a predominance of plums. Indeed, plums were the most frequent accessions collected at this municipality. Unfortunately, only two local plums from this location were described by Herrero (1964), and none of them shared a name with the ones analyzed in this paper. Special attention was paid by Herrero (1964) to the ‘Almagreño’ peach from Galaroza, described as a landrace in which many trees were seedlings. This is also consistent with the great diversity observed in this work among all the accessions sharing the
‘Almagreño’ name (Figure 2). Cherries from Galaroza, mentioned by the same author, were apparently not grafted trees but selected spontaneous seedlings from the base of isolated trees. This observation is not consistent with the similarity of all ‘Burral’ accessions that are alike, but it may explain the differences observed between ‘Aranjuez’ 1 and 2, ‘Tierno’ 1 and 2, and among ‘Blandillo’ accessions from Constantina.

In conclusion, the use of highly transferable SSR loci is particularly interesting in genetic resource surveys for different plant species, such as the one described in this work. These multi-species surveys are very efficient for horticultural products, such as fruit trees, since traditional farmers usually conserve a moderate number of trees from many different species and cultivars in the same orchard. In this work, we confirmed the usefulness of a single set of seven polymorphic SSRs transferable across the genus *Prunus*, allowing the initial characterization of apricot, plum, peach, and cherry accessions. The results obtained show the wide genetic variability of the collected sample, which highlights the need to preserve traditional cultivars, as they are important genetic resources for future breeding programs. *In-situ* and *ex-situ* conservation strategies should be applied as well as further characterization of these plant materials including morphological traits and a set of reference cultivars for each species. An initial collection of part of this plant material (28 accessions; see Supplementary Table B) is currently preserved in the US and will be transferred to the Spanish germplasm banks of different *Prunus* species.

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**REFERENCES**


تنوع زنتیکی کولتیوارهای میوه های هسته دار که در مزارع جنوب اسپانیا نگهداری می شود

چکیده

کولتیوارهای سنتی و قدیمی منابع زنتیکی با ارزشی برای اصلاح و بهبود گیاهان به شمار می روند ولی تعداد زیادی از آنها در قرن اخیر از میان رفته اند. هدف این پژوهش شناسایی و یزیگی های کولتیوارهای سنتی گونه های مختلف Prunus واقع در باغ های خانگی کوچک در جنوب غربی اسپانیا و ارزیابی تنوع زنتیکی و روابط آنها با هم بود. این مطالعه با استفاده از 8 جایگاه ژنی SSR قابل انتقال در سرای جنس Prunus، 12 نمونه نتیجه که به 36 عنوان کولتیور سنتی تعلق داشت مورد تحلیل قرار گرفت. موجب پدیده تنوع جایگاه ها (Loci) برای تحلیل جایگاه های جنس (جنس) Prunus و بررسی ژنتیکی که ارزشی های مختلف شناسایی شد که امکان وجود همنام ها (homonym) را در اساسی کولتیوارهای سنتی آشکار می کرد. نتایج تحلیل خوش ای با طبقه بنی اند، مطالعه های مبتنی بر مداخلات ژنتیک در داخل هر گونه مهم و بود. مباحث پژوهش حاکی از تغییرات زنتیکی گسترده در کولتیوارهای سنتی میوه های هسته دار کاچش شده در باغ های خانگی کوچک بود و این امر مورد حفاظت از آنها را با استفاده از روش های مختلف در محل رویش و یا خارج از محل اولیه رویش. در Sevilla، 28 نمونه از نمونه های نتیجه که به 36 عنوان کولتیور سنتی می بود. مربی های مقیم در خارج از محل اولیه رویش در دانشگاه در اسپانیا نگهداری می شود. در SSR بررسی ها و مطالعات چند-گونه ای در مزارع، استفاده از ژاکت گونه ای به راحتی با تأثیرات بالایی دارند، روشن کارآمدی است.