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

P. Rallo[1]*, M. Rocío Jiménez[1], L. Casanova[1], A. Morales-Sillero[1], and M. Paz Suárez[1]

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).

[1] Department of Agroforestry Sciences, ETSIA, University of Seville, Carretera de Utrera, km 1, 41013 Seville, Spain.
*Corresponding author; e-mail: prallo@us.es

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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 $(NH_4)_2SO_4$], 0.20 mM dNTPs, 4.00 mM $MgCl_2$, 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 (Gene Amp® 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.

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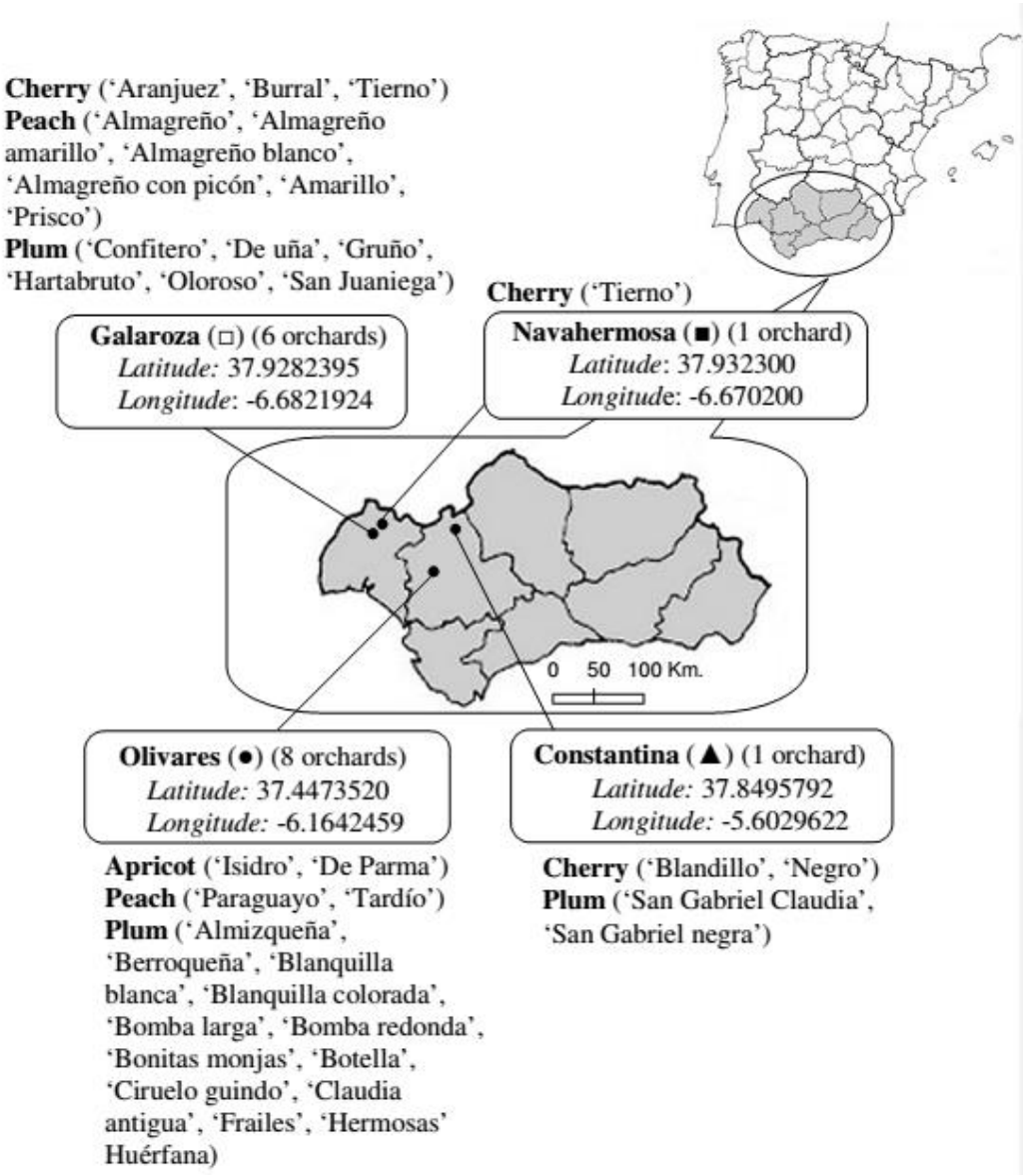

Figure 1. Geographic location of the municipal districts where on-farm exploration occurred. The number of orchards and the names of the traditional cultivars found per species are indicated for each municipal district.

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= $1-\Sigma p_i^2$, where p_i is the frequency of the *i*th allele) and the

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Table 1. Cultivar accessions of various *Prunus* species analyzed in this study.

Species	Cultivar, accession	Municipal district (Province)	Orchard name
Apricot	Isidro 1, 2	Olivares (Sevilla)	La Era 2
Prunus armeniaca L	Isidro 3, 4	Olivares (Sevilla)	Huerta Cachón
	De Parma 1, 2, 3	Olivares (Sevilla)	Huerta Macario 1
Cherry	Aranjuez 1, 2	Galaroza (Huelva)	Los Roblecillos
Prunus avium L.	Burral 1	Galaroza (Huelva)	La Confesa 1
	Burral 2	Galaroza (Huelva)	La Confesa 2
	Burral 3, 4	Galaroza (Huelva)	Los Roblecillos
	Blandillo 1, 2, 3, 4, 5, 6, 7, 8, 9	Constantina (Sevilla)	San Gabriel
	Negro 1, 2, 3, 4	Constantina (Sevilla)	San Gabriel
	Tierno 1	Navahermosa (Huelva)	Unnamed
	Tierno 2	Galaroza (Huelva)	Los Roblecillos
Peach	Almagreño	Galaroza (Huelva)	Los Roblecillos
Prunus persica L.	Almagreño amarillo sin picón 1, 2, 3, 4	Galaroza (Huelva)	La Confesa 1
	Almagreño blanco 1	Galaroza (Huelva)	La Confesa 1
	Almagreño blanco 2	Galaroza (Huelva)	Huerta Venecia
	Almagreño blanco 3, 4	Galaroza (Huelva)	Huerta río Múrtigas
	Almagreño con picón	Galaroza (Huelva)	La Confesa 1
	Amarillo 1, 2	Galaroza (Huelva)	La Confesa 1
	Amarillo 3	Galaroza (Huelva)	Huerta Venecia
	Paraguayo 1	Olivares (Sevilla)	Huerta peluquero
	Paraguayo 2	Olivares (Sevilla)	Huerta Macario 1
	Prisco 1, 2	Galaroza (Huelva)	Huerta Venecia
	Tardío o menudillo 1, 2	Olivares (Sevilla)	Huerta Macario 2
	Tardío o menudillo 2	Olivares (Sevilla)	Huerta Macario 2
European Plums	Almizqueña 1, 2, 3	Olivares (Sevilla)	Huerta Macario 1
Prunus domestica L.	Berroqueña 1, 2, 3	Olivares (Sevilla)	La Era 2
	Berroqueña 4, 5, 6	Olivares (Sevilla)	Huerta Macario 1
	Blanquilla blanca 1, 2	Olivares (Sevilla)	La Era 2
	Blanquilla blanca 3	Olivares (Sevilla)	Huerta Cachón
	Blanquilla coloradas 1	Olivares (Sevilla)	Huerta Macario 1
	Blanquilla coloradas 2	Olivares (Sevilla)	Huerta Cachón
	Bomba larga 1, 2	Olivares (Sevilla)	Huerta Macario 1
	Bonitas monjas 1	Olivares (Sevilla)	Huerta Macario 1
	Bonitas monjas 2, 3	Olivares (Sevilla)	Huerta Cachón
	Botella 1, 2	Olivares (Sevilla)	La Era 1
	Botella 3	Olivares (Sevilla)	Huerta Macario 1
	Botella 4	Olivares (Sevilla)	Los Rubiales
	Claudia antigua 1	Olivares (Sevilla)	La Era 2
	Claudia antigua 2, 3	Olivares (Sevilla)	Huerta Macario 1
	Confitero 1, 2, 3, 4	Galaroza (Huelva)	Francisco "colorao"
	De uña 1, 2, 3, 4, 5	Galaroza (Huelva)	Huerta Venecia
	Frailes 1, 2, 3	Olivares (Sevilla)	Huerta Macario 1
	Gruño 1, 2, 3	Galaroza (Huelva)	Los Roblecillos
	Hermosas 1, 2, 3	Olivares (Sevilla)	Huerta Macario 1
	Huérfana 1, 2	Olivares (Sevilla)	El Injertal
	Huérfana 3	Olivares (Sevilla)	Huerta Cachón
	Oloroso 1	Galaroza (Huelva)	Huerta Venecia
	Oloroso 3	Galaroza (Huelva)	Los Roblecillos
	San Gabriel claudia 1, 2	Constantina (Sevilla)	San Gabriel
	San Gabriel negra 1, 2	Constantina (Sevilla)	San Gabriel
	Sanjuaniega 1, 2, 3, 4	Galaroza (Huelva)	La Confesa 1
	Sanjuaniega 5	Galaroza (Huelva)	Los Roblecillos
Other plums	Bomba redonda 1, 2	Olivares (Sevilla)	Huerta Macario 1
	Ciruelo guindo 1, 2	Olivares (Sevilla)	Huerta Cachón
	Hartabruto 1, 2	Galaroza (Huelva)	Los Roblecillos
	Oloroso 2	Galaroza (Huelva)	Huerta Venecia

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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).

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

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 *He* 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 s*pecies (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 58 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.

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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).

Species	SSR locus	Number of genotypes	Range size bp	Number of alleles	*Ho*	*He*	*PD*
Apricot	ps02a12	2	(154-184)	3	1	0.62	0.49
	UDP98-410	2	(112-118)	2	0.43	0.34	0.49
	UDP98-409	2	(139-164)	3	0.57	0.65	0.49
	UDP96-005	2	(109-144)	4	1.00	0.75	0.49
	BPPCT-002	2	(187-191)	3	1	0.65	0.49
	BPPCT-004	1	(196)	1	0	0	0
	BPPCT-010	2	(120-124)	2	0.57	0.41	0.49
	BPPCT-026	1	(131-144)	2	1	0.5	0
	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
	BPPCT-026	7	(133-146)	6	0.05	0.77	0.81
	Mean			3.75	0.18	0.47	0.50
Plum[a]	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	21	(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			14.25	0.93		0.91

[a] Plums were excluded from *He* calculations due to their polyploid nature.

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

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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

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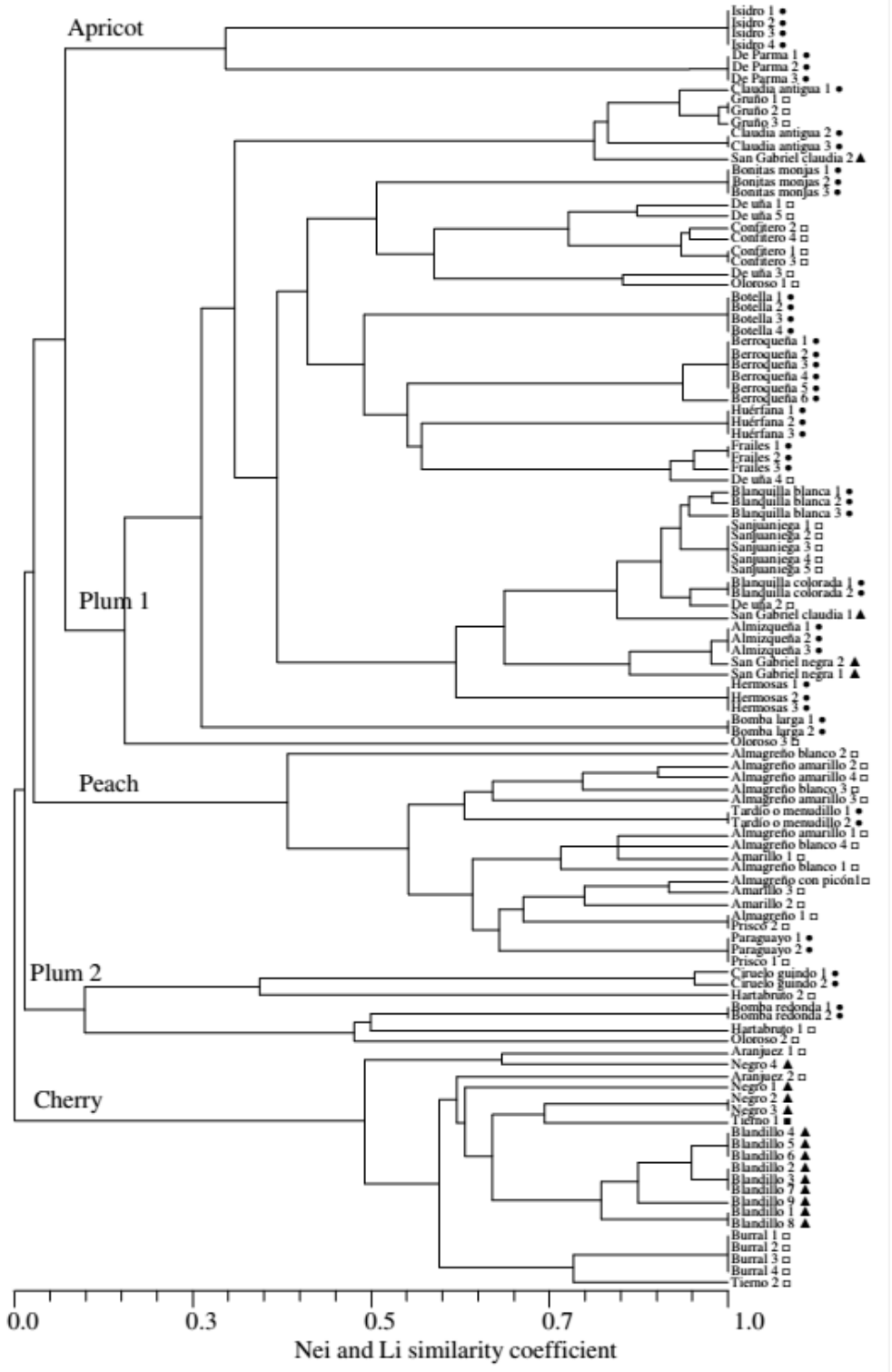

Figure 2. Dendrogram of 112 accessions of local stone fruit cultivars based on UPGMA analysis of seven SSR loci (BPPCT-026 not included). Symbols refer to the geographic locations of the collected accessions [Constantina (▲), Galaroza (□), Navahermosa (■), Olivares (●)].

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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

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

پ. رالو، م. روسیو جیامنز، ل. کاسانوا، ا. مورالز-سیلرو، و م. پز سوارز

چکیده

کولتیوارهای سنتی و قدیمی منابع ژنتیکی 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 ویژگی های کولتیوارهای سنتی گونه های مختلف *Prunus*واقع در باغ های خانگی کوچک در جنوب غربی اسپانیا و ارزیابی تنوع ژنتیکی و روابط آنها با هم بود. به این منظور، با استفاده از ۸ جایگاه ژنی *SSR* قابل انتقال در سراسر جنس *Prunus*، ۱۲ نمونه ثبت شده که به ۳۶ عنوان کولتیوار سنتی تعلق داشت مورد تحلیل قرار گرفت. مفید ترین جایگاه ها (Loci) برای تحلیل گونه های جنس *Prunus*شامل UDP96-005، BPPCT-002، UDP98-410و ps02a12 بود. در مجموع، ۱۵۲ آلل مورد مشاهده قرار گرفت و ۱۱۲ تای آنها اختصاصی گونه های معینی بودند. میان آنها، شصت و هشت ژنوتیپ مختلف شناسایی شد که امکان وجود همنام ها (*homonym*) را در اسامی کولتیوارهای سنتی آشکار می کرد. نتایج تحلیل خوشه ای با طبقه بندی رده بندی علمی (taxonomic classification) گونه های مطالعه شده و مبادی جغرافیایی نمونه های ثبت شده در داخل هر گونه همخوان بود. نتایج پژوهش حاکی ازتغییرات ژنتیکی گسترده در کولتیوارهای سنتی میوه های هسته دار کاشته شده در باغ های خانوادگی کوچک بود و این امرضرورت حفاظت از آنها را با استفاده از رهیافت های حفاظت در محل رویش و در خارج از محل اولیه رویش(*ex-situ*) روشن می کند. ۲۸ نمونه از نمونه های ثبت شده مزبور هم اکنون در خارج از محل اولیه رویش در دانشگاه Sevilla در اسپانیا نگهداری می شود. در بررسی ها ومطالعات چند-گونه ای در مزارع، استفاده از SSR هایی که قابلیت انتقال بالایی دارند، روش کارآمدی است.

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