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Genetic variability among local apricots (*Prunus armeniaca* L.) from the Southeast of Spain

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Abstract

The fast rotation of new cultivars demanded by modern fruit growers implies the loss of many old varieties with valuable characters. Then, the need arises to keep and characterize this germplasm for future breeding projects. The region of Murcia, together with Valencia, in the East and Southeast of Spain respectively, are important and ancient producers of apricot (*Prunus armeniaca* L.), and many local cultivars have appeared and diversified in this area. A collection of 28 of these old cultivars, plus eight clonal selections of the cultivar 'Búlida', is maintained at the Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA, Murcia, Spain). In order to characterize their genetic diversity and to identify the collection with molecular markers, 17 microsatellite primers pairs were used. Thirteen of these primers produced polymorphic repeatable amplification patterns, and 31 genotypes were identified among the 36 apricot accessions. In addition to this, an evaluation of the genetic diversity found in the field within the cultivar 'Búlida' was made, the predominant cultivar for the canning industry in the region. For this, 66 field samples were analyzed with seven microsatellite markers. The results suggest that all the samples could derive from four closely-related genotypes, one of them accounting for 89% of the samples.

Additional key words: cultivar identification, genetic relationships, microsatellites, molecular diversity, molecular markers.

Resumen

Variabilidad genética entre cultivares de albaricoquero tradicionales (*Prunus armeniac*a L.) del sureste español

La rápida rotación de nuevas variedades que exige la fruticultura moderna, implica la pérdida de muchas variedades antiguas con caracteres potencialmente valiosos. En consecuencia, surge la necesidad de mantener y caracterizar este germoplasma para futuros proyectos de mejora. La región de Murcia, junto con la de Valencia, en el este y sureste de España respectivamente, son importantes y antiguas productoras de albaricoque (*Prunus armeniaca* L.), y muchos cultivares autóctonos han aparecido y se han diversificado en la zona. En el Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA, Murcia, España) se mantiene una colección de 28 de estas antiguas variedades más 8 selecciones clonales del cv. 'Búlida'. Para caracterizar molecularmente la diversidad e identidad genética de esta colección, se emplearon 17 marcadores microsatélite. En 13 de ellos se detectaron pautas de amplificación polimórficas y reproducibles, y se pudieron identificar 31 genotipos. Además de la evaluación de la colección, se planteó la evaluación de la diversidad genética en campo del cv. 'Búlida', que es la predominante para uso en la industria conservera de la región. Para ello se analizaron 66 muestras de campo con 7 marcadores microsatélite. Los resultados sugieren que todas las muestras de campo podrían derivar de cuatro genotipos estrechamente relacionados, agrupando uno de ellos al 89% de las muestras.

Palabras clave adicionales: diversidad molecular, identificación de cultivares, marcadores moleculares, microsatélites, relaciones genéticas.

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Abbreviations used: AF (allele frequency), AFLP (amplified fragment length polymorphism), AS (allele size), IMIDA (Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario), PD (power of discrimination), PPV (*Plum Pox Virus*), RAPD (random amplified polymorphic DNA), RFLP (restriction fragment length polymorphism), SCAR (sequence characterized amplified region), SSR (simple sequence repeat), UPGMA (unweighted pair-group method with arithmetic mean).

Introduction

The cultivated apricot, *Prunus armeniaca* L. (Rosaceae, subfamily Prunoidae), is the third most important species of the stone-fruit crops. It is distributed worldwide, but most of the commercial production is concentrated in the Mediterranean area - this accounts for more than 55% of the world production. This area, together with other important producers such as Pakistan, Ukraine, China, Iran and the U.S.A., forms up to 80% of the global production (FAOSTAT Agriculture Database 2001).

Apart from *P. armeniaca,* other minor species are also included under the general denomination of apricot, namely *P. mandshurica* (Maxim.) Koehne, *P. sibirica* L. and *P. mume* (Sieb.) Sieb et Zucc. All of them are interfertile, diploid species with eight pairs of chromosomes (2n = 16) which include self-compatible and self-incompatible cultivars. A high pomological and genetic diversity is usually recognized within *P. armeniaca*, which has led to the separation of at least four major eco-geographical groups: Central Asian (Afghanistan, Baluchistan, Pakistan), Irano Caucasian (Caucasus, Iran, Iraq, Syria, Turkey, North Africa), Dzungar-Zailig (Kazakhstan, Xinjiang) and European (Europe, North America, South Africa, Australia) (Kostina, 1969). Other groups and sub-groups are also recognized, such as East Chinese and North Chinese (Layne *et al.*, 1996). The European group is the most recent and the least variable, composed mainly of self-compatible genotypes. It includes most of the commercial cultivars grown in Europe and America.

The origin of the apricot is in Central Asia and China, from there it was probably introduced into Europe through Greece (400 BC), and also later (100 BC) by the Romans (Bailey and Hough, 1975). In Spain, the main apricot growing areas are located in the neighbouring regions of Murcia and Valencia, in the Southeast of Spain. Spanish cultivars are thought to originate from the confluence of two different eco-geographical groups. One of these would be the Irano Caucasian, introduced by the Arabs and composed of self-incompatible cultivars with lower chilling requirements and small and precocious fruits. The other component, the European group, is composed of self-compatible cultivars with high chilling requirements and big and late-ripening fruits (Crossa-Raynaud, 1961; Egea *et al*., 1988).

In Murcia and Valencia, a high number of local varieties have been selected through the centuries by growers, from a genetic pool that is undifferentiated and highly diverse. Apricot has a low plasticity in its adaptability to different edaphoclimatic conditions, being highly specific in its ecological requirements (Layne *et al*., 1996). As a consequence, many cultivars have been selected that are apt only in very limited geographical niches. The area of the upper basin of the Segura river, in Murcia, with many alluvial terraces with varied orientations and very apt for apricot cultivation, is the origin of many of these local selections, and at least 76 denominations were recorded in a survey in Murcia in the 1980s (Martínez-Cutillas and Gómez, 1983). An interesting sub-group of cultivars originating in Murcia is the one collectively known as «Clases». These cultivars have a low canning aptitude, but have a fruit size and organoleptic characteristics that make them excellent for table use and export when fresh (Martínez-Valero, 1981). In spite of their good quality, most of the cultivars of this group are self-incompatible, which has contributed to the decline of their cultivation. However, it is important to conserve and study this genetic pool in order to preserve characters that confer high quality.

Another apricot cultivar in the region of Murcia is 'Búlida', which is the cultivar of choice for the canned fruit market. It was selected from the genetic pool of the region (Egea *et al*., 1988) at the beginning of the development of the canning industry, but in contrast to other local cultivars, it was propagated vegetatively and its characters are more stable and homogeneous. Previous surveys of the pomological diversity of this cultivar in Murcia have been performed (Piñero *et al*., 2006) and the results have detected variation that has prompted the interest in obtaining clonal selections. As a consequence, it is relevant to evaluate the diversity of the genetic pool on which the selection is carried out, and verify if that pomological variation has a genetic base.

In order to perform the assessment of the genetic diversity of the germplasm of cultivated plants, both in collections and in the field, the use of molecular markers has become essential. The analysis of DNA with these genetic tools allows a level of resolution in the identification of genotypes that is impossible to obtain with morphological observation alone. Many of these genetic markers have been developed along the years, and from an early phase many of them have been applied to the genus *Prunus*, starting with the construction of a genetic map for improving breeding selection in peach (Chaparro *et al*., 1994). At least three genetic linkage maps of apricot have been published

already (Hurtado *et al.,* 2002; Vilanova *et al*., 2003; Lambert *et al*., 2004). Apricot diversity and genetic relationships have been studied using isozymes (Byrne and Littleton, 1989; Badenes *et al*., 1996), restriction fragment length polymorphisms (RFLPs) (De Vicente *et al*., 1998), random amplified polymorphic DNA (RAPDs) (Badenes *et al*., 2000), amplified fragment length polymorphisms (AFLPs) (Hagen *et al*., 2002; Hurtado *et al.,* 2002) and sequence characterised amplified regions (SCARs) (Mariniello *et al*., 2002). However, most of these early types of molecular markers have been displaced by others, such as microsatellites (simple sequence repeat, SSR) - which are co-dominant and offer significant advantages in terms of reproducibility and simplicity (Morgante and Olivieri, 1993). There is already a considerable literature related to the use of microsatellites in the study of genetic relationships in apricot (Hormaza, 2001, 2002; Romero *et al*., 2003; Zhebentyayeva *et al*., 2003; Sánchez-Pérez *et al.,* 2004; 2006; Krichen *et al*., 2006; Tian-Ming *et al*., 2007). However, most of the work has been done with apricot varieties of very diverse origin, and many local Spanish varieties have not been analysed yet.

The present work was started with two separate objectives. The first was to analyse the diversity and genetic relationships among 28 cultivars of apricot and eight clonal selections of 'Búlida', all of them traditional from the regions of Murcia and Valencia and many of them no longer in use, maintained at the germplasm collection of the IMIDA. The second objective was to analyse the genetic molecular diversity of the genetic pool of the cultivar 'Búlida' in its cultivation area in Murcia. In both experiments, the markers of choice were the microsatellites. The loci analysed were previously located and developed in peach (Cipriani *et al.,* 1999; Sosinski *et al*., 2000; Testolin *et al*., 2000; Dirlewanger *et al.,* 2002), but previous work by Hormaza (2002) demonstrated their suitability for apricot.

Material and methods

Plant material

The work was structured in two separate experiments and, as a consequence, two different sets of plant samples were used. In the experiment for the analysis of the genetic relationships of the cultivars of the IMIDA germplasm collection, 28 traditional Spanish apricot cultivars were analysed. In this experiment, a set of eight clonal selections of the cultivar 'Búlida', obtained at the IMIDA, was also included. The codes, local names, place of origin and main agronomic characteristics of these cultivars are listed in Table 1.

For the analysis of the genetic diversity of the cultivar 'Búlida' in the field, a total of 66 trees of 'Búlida' were sampled at 10 different locations in Murcia where this cultivar is grown (Table 2). The total number of apricot samples analysed was 102.

DNA extraction and PCR amplification

Total genomic DNA was isolated from young fresh leaves using the procedure described by Hormaza (2002). Extracted DNA was quantified and diluted to 10 ng μL^{-1} final concentration and used for PCR amplification. Seventeen microsatellite primers (SSRs), originally developed for peach and representing different regions of the peach genome, were used for the molecular analysis (Table 3). The forward primer of each pair was labelled using the CY5 fluorophore (Sigma, UK). PCR reactions were performed in a 12 µL volume, and the reaction mixture contained 67 mM Tris-HCl pH 8.8, 16 mM $(NH_4)_2$ SO_4, 0.1% Tween-20, 2.5 mM $MgCl_2$, 0.96 mM of each dNTP, 0.2 µM of each primer (except for pchgms and pchcms primers for which the reaction mixture contained 0.12 µM of each primer), one unit of *Taq* DNA polymerase (Ecogen, Barcelona) and 20 ng of genomic DNA. PCR reactions were carried out in a GeneAmp-9600 thermocycler (Applied Biosystems, CA, USA). The amplification program consisted of 5 min at 95°C, 35 cycles of 45 s at 94°C, 45 s at 57°C and 45 s at 72°C, followed by an extension cycle of 10 min at 72° C. Amplified DNA fragments were separated by electrophoresis in 8% acrylamide/bisacrylamide agarose gels in 1× TBE buffer (ReproGel™ High Resolution, Amersham Pharmacia Biotech, Uppsala) in an automatic sequencer ALFexpress® II DNA Analyser (Amersham Pharmacia Biotech). Amplified DNA fragments were visualised, scored and analysed with ALFwin Fragment Analyser 1.00 software (Amersham Pharmacia Biotech). At least two independent SSR reactions were performed for each DNA sample.

Data analysis

The information of each microsatellite loci was estimated by use of allele number per locus (*Na*) and

Table 1. The 36 traditional Spanish apricot cultivars maintained at the IMIDA collection and included in this study

Code	Cultivar	Origin	Skin colour	Flesh colour	Ripeness date[a]	Ho[b]
A.07.92	Blanco	Valencia	White	White	E	0.46
A.22	A.22	Murcia (Blanca)	Yellow-Orange	Orange	I	0.77
A.10.92	Canino	Valencia (Sagunto)	Orange	Orange	I	0.62
A.30	Cañahueca[c]	Murcia (Cieza)	Yellow	Yellow honey	I	0.8
A.62	Carrascal[c]	Murcia	Yellow-Orange	Yellow-Orange	I	0.69
A.2	Carrichosa 2 × 5	Murcia (Cieza)	Sweet Green	Yellow-Orange	I	0.58
A.52	Colorao Antón[c]	Murcia	Yellow-Orange	Yellow	I	0.62
A.46	Cortos Archena	Murcia	Yellow Cream	Yellow-Orange	I	0.96
A.04.92	Cristalí	Valencia	White	White	I	0.77
A.48	Eugenios[c]	Murcia	Sweet Orange	Sweet Orange	I	0.62
FR	Fermín Rojo	Murcia	Yellow-Orange	Orange	I	0.62
A.33	Gitano[c]	Murcia (Abarán)	Yellow-Orange	Yellow-Orange	I	0.69
A.19	Liberato[c]	Murcia (Cieza)	SweetYellow	Yellow-Orange	I	0.62
A.01.92	Martinet	Valencia	Yellow	Yellow Cream	L	0.62
A.12	Mauricio	Murcia (Archena)	Yellow-Orange	Sweet Orange	E	0.62
A.03.92	Mitger	Castellón	Yellow Cream	Yellow Cream	I	0.62
A.27	Moniquí Fino[c]	Murcia	White Cream	White	L	0.69
A.61	Mosós	Valencia	Yellow Cream	Orange	L	0.77
A.26	Ojaíco[c]	Murcia (Abarán)	Yellow Cream	Yellow-Orange	I	0.77
A.58	Pacorros Archena	Murcia	Yellow Cream	Yellow-Orange	L	0.85
A.67	Pelícano Archena	Murcia	Yellow-Green	Yellow-Orange	I	0.77
A.70	Pepito Blancos[c]	Murcia	Yellow Cream	White	I	0.69
A.53	Pepito del Rubio[c]	Murcia	Yellow Cream	White	I	0.62
A.31	Pericales[c]	Murcia	Yellow-Green	Orange	I	0.62
A.43	Real Fino	Murcia (Pliego)	Yellow Cream	White	L	0.85
A.68	Tadeo	Valencia	SweetYellow	Yellow-Orange	L	0.83
A.9	Valenc. Glorieta	Valencia	Sweet Orange	Orange	E	0.77
A.20	Velázquez[c]	Murcia (Cieza)	Yellow-Green	Yellow-Orange	I	0.54
A1387[d]	Búlida clonal selection	Murcia (Calasparra)	Yellow-Orange	Orange	I	0.54
A5000	Búlida clonal selection	Murcia (Calasparra)	Yellow-Orange	Orange	I	0.62
A4500	Búlida clonal selection	Murcia (Calasparra)	Yellow-Orange	Orange	I	0.54

[a] E-Early; I-Intermediate; L-Late. [b] Ho: observed genetic heterozygosity. [c] The accessions that belong to the «Clases» group. [d] A1387, A4800, A1087, A1587 A1687 and A1287 accessions showed the same genotype at 13 SSR loci.

effective number of alleles per locus (*Ne*), calculated as $1/\Sigma p_i^2$ where p_i is the frequency of the *i*th allele. The observed genetic heterozygosity (*Ho*) of apricot genotypes was calculated as the number of heterozygous loci for a given cultivar divided by the total number of loci assayed. Genotypes showing a single amplified fragment were considered as homozygous for that particular locus since segregation analysis is needed to detect the presence of putative null alleles (Callen *et al*., 1993). The observed genetic heterozygosity (*Ho*) of each SSR marker was calculated as the number of heterozygous genotypes divided by the total number of genotypes. Expected genetic heterozygosity (*He*) was calculated as $1-\Sigma p_i^2$, where p_i is the frequency of the i^{th} allele (Nei, 1973). Wright`s fixation index (F = 1-*Ho/He*) was used to compare both heterozygosities (Wright, 1951). The ability of a marker to discriminate between two random cultivars was estimated for each locus with the power of discrimination (PD), which was calculated as $1-\Sigma g_i^2$, where g_i is the frequency of i^{th} genotype (Kloosterman *et al.,* 1993). These analyses were computed with the GeneAlEx V5 program (Peakall and Smouse, 2001). Genetic variation among the studied apricot cultivars was estimated and a dendrogram was constructed using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) method and as an estimation of genetic similarity, it was used the Nei distance D_A (Nei *et al*., 1983) with the Populations 1.2.28 program (http://www.cnrs.gif.fr/pge) (Langella, 1999). The dendrogram was drawn with the Molecular Evolutionary Genetics Analysis (MEGA) Program v. 2.1 (Kumar *et al*., 1993).

Table 2. The 66 field samples of apricot 'Búlida' cultivated in different areas of Murcia

Code	Local area	Code	Local area
AR 1-3	Archena	CRV 452	Caravaca
CA 312	Calasparra	CRV 453	Caravaca
CA 313	Calasparra	CRV 454	Caravaca
CA 324	Calasparra	M 121	Mula
CA 331	Calasparra	M 122	Mula
CA 332	Calasparra	M 123	Mula
CA 333	Calasparra	M 131	Mula
CE 213	Ceutí	M 132	Mula
CE 215	Ceutí	M 134	Mula
CE 221	Ceutí	M 135	Mula
CE 222	Ceutí	MOL 251	Molina
CE 223	Ceutí	MOL 252	Molina
CE 224	Ceutí	MOL 253	Molina
CE 225	Ceutí	MOL 255	Molina
CE 231	Ceutí	MOL 311	Molina
CE 232	Ceutí	MOL 312	Molina
CE 233	Ceutí	MOL 313	Molina
CE 241	Ceutí	MRT 322	Moratalla
CE 242	Ceutí	MRT 323	Moratalla
CE 243	Ceutí	MRT 324	Moratalla
CE 244	Ceutí	PL 141	Pliego
CEH 412	Cehegín	PL 142	Pliego
CEH 413	Cehegín	PL 143	Pliego
CEH 414	Cehegín	PL 151	Pliego
CEH 421	Cehegín	PL 152.1	Pliego
CEH 422	Cehegín	PL 152.2	Pliego
CEH 423	Cehegín	PL 154	Pliego
CEH 431	Cehegín	PL 155	Pliego
CEH 432	Cehegín	UL 1-1	Ulea
CEH 434	Cehegín	UL 1-2	Ulea
CEH 435	Cehegín	UL 1-3	Ulea
CRV 441	Caravaca	UL 1-4	Ulea
CRV 442	Caravaca	Balbino[a]	Ulea

[a] Balbino is a clonal selection from Ulea.

Results and discussion

Polymorphism and heterozygosity of SSR markers

Seventeen SSR primer pairs, developed for peach and representing different regions of the peach genome (Table 3), were tested in 36 accessions of apricot from the IMIDA collection. The 17 primer pairs had different levels of amplified bands the size of which ranged from 83 to 266 bp, in the same size range as those reported in related species (Sosinski *et al*., 2000; Testolin *et al*., 2000; Hormaza, 2002). Four pairs failed to reveal any variation in the accessions tested (pchcms2, UDP96-003, UDP96-018 and UDP97-403) and thirteen pairs amplified polymorphic markers (76%). The genotypes obtained for the 13 polymorphic loci allowed the distinction of 31 genotypes among the 36 accessions. The allelic distribution at polymorphic microsatellite loci was analysed in the apricot samples from which redundant genotypes had been excluded (Table 4). The number of alleles observed (*Na*) at each locus ranged from two (BPPCT030, pchgms4 and UDP97-402) to six (UDP96-005) with an average of four alleles per locus. Altogether, 47 alleles were identified in the set of accessions. In all samples, the effective number of alleles was lower than observed and varied from 1.101 for UDP97-402 to 3.571 for UDP96-010 (Table 4). These differences between the number of effective and observed alleles indicate the presence of rare alleles that exist in a few genotypes and could be used for their identification (Table 4).

The observed heterozygosity ranged from 1.0 for BPPCT004, BPPCT008 and UDP96-005 to 0.032 for pchgms3, with a mean of 0.677, and was higher than the expected heterozygosity in 12 loci (Table 4). Consequently, the fixation index (F) values were negative for all the loci used except for pchgms3 locus, indicating an excess of heterozygosity. Negative F values could indicate that the global behaviour of the apricot genotypes studied was similar to an assortative mating or selection. The most informative locus was UDP96-010, with a PD of 0.778, and the least informative loci were BPPCT033 with a PD of 0.127. The average of this parameter for all loci was 0.523. The number of genotypes detected at each locus ranged from two (BPPCT008, BPPCT030 and UDP97-402) to seven (UDP98-406), with an average of four genotypes per locus (Table 4). Nine alleles at seven loci showed frequencies lower than 0.05 (Table 5), whereas six alleles at six loci showed frequencies higher than 0.50 (BPPCT030-148; BPPCT033-150; pchgms2-157; pchgms3-195; pchgms4-176; UDP97-402-146) and among them, two (BPPCT030-148; UDP97-402-146) were nearly fixed with frequencies higher than 0.90 (Table 5).

The results confirm that peach SSR markers can be used to identify the level of genetic variability in apricot, in according with the findings of other authors (Hormaza 2001, 2002; Romero *et al*., 2003; Zhebentyayeva *et al*., 2003; Sánchez-Pérez *et al*., 2004, 2006; Krichen *et al*., 2006; Tian-Ming *et al*., 2007). The level of polymorphism observed in apricot using SSR markers was higher than for other, previously-studied markers (Byrne and Littleton, 1989; Badenes *et al*., 1996, 2000; de Vicente *et al*., 1998; Hagen *et al*., 2002).

Table 3. The 17 peach SSR sequences assayed and polymorphism obtained in the apricot cultivars studied

Locus	Linkage group	Size range (bp)	Polymorphism	Reference
BPPCT004	2	190-206	Yes	Dirlewanger *et al.* (2002)
BPPCT008	6	92-110	Yes	Dirlewanger *et al.* (2002)
BPPCT030	2	148-152	Yes	Dirlewanger *et al.* (2002)
BPPCT033	8	142-158	Yes	Dirlewanger *et al.* (2002)
pchcms2	7	172	No	Sosinski *et al.* (1999)
pchcms5	6	214-266	Yes	Sosinski *et al.* (1999)
pchgms1	2	138-166	Yes	Sosinski *et al.* (1999)
pchgms2	4	145-171	Yes	Sosinski *et al.* (1999)
pchgms3	1	171-197	Yes	Sosinski *et al.* (1999)
pchgms4	5	150-176	Yes	Sosinski *et al.* (1999)
UDP96-003	4	97	No	Cipriani *et al.* (1999)
UDP96-005	1	106-150	Yes	Cipriani *et al.* (1999)
UDP96-010	6	83-103	Yes	Cipriani *et al.* (1999)
UDP96-018	1	246	No	Cipriani *et al.* (1999)
UDP97-402	2	130-146	Yes	Cipriani *et al.* (1999)
UDP97-403	3	120	No	Cipriani *et al.* (1999)
UDP98-406	2	87-105	Yes	Cipriani *et al.* (1999)

Genetic relationships within the IMIDA apricot collection

Thirteen polymorphic SSR loci were scored in 36 IMIDA apricot accessions (Tables 1 and 4). No polymorphism at the DNA level was detected between the A1387, A1287, A4800, A1087, A1587 and A1687 accessions of the 'Búlida' clonal selection. Therefore, this analysis allowed the distinction of 31 genotypes among the 36 accessions studied (Annexe 1). Genetic heterozygosity of apricot cultivars ranged between 0.46 ('Blanco') and 0.96 ('Cortos Archena'), with an average value of 0.53 (Table 1). Six privative alleles were found at four loci (BPPCT033-142 in 'Carrichosa'; pchgms2-149 in 'Cristalí'; both pchgms3-171 and pchgms3-181 in 'Pepito Blanco'; UDP96-005-138 in 'Mauricio'; UDP96-005-150 in 'Blanco'); therefore, these loci could be very useful for genotype identification. The five

Table 4. Genetic parameters for 13 peach SSR loci in the 36 apricot IMIDA accessions

Locus	Na	Ne	Ho	He	F	PD	Genotypes
BPPCT004	4	3.056	1.000	0.673	–0.486	0.658	4
BPPCT008	3	2.381	1.000	0.580	–0.724	0.320	2
BPPCT030	2	1.174	0.161	0.148	–0.088	0.271	2
BPPCT033	3	2.062	0.967	0.515	–0.877	0.127	3
pchcms5	5	3.022	0.969	0.669	–0.446	0.720	6
pchgms1	4	2.696	0.871	0.629	–0.385	0.647	5
pchgms2	4	1.715	0.516	0.417	–0.238	0.635	4
pchgms3	4	1.992	0.032	0.498	0.935	0.497	3
pchgms4	2	1.875	0.548	0.467	–0.175	0.564	3
UDP96-005	6	3.069	1.000	0.674	–0.483	0.656	6
UDP96-010	4	3.571	0.897	0.720	–0.245	0.778	6
UDP97-402	2	1.101	0.097	0.092	–0.051	0.175	2
UDP98-406	4	3.331	0.742	0.700	–0.060	0.749	7
Total	47	31.045	8.799	6.798	–3.324	6.796	53
Average/locus	4	2	0.677	0.522	–0.256	0.523	4

Na: allele number per locus. Ne: effective number of alleles per locus. Ho: observed genetic heterozygosity. He: expected genetic heterozygosity. F: fixation index. PD: power of discrimination.

Table 5. Allele size (AS, bp) and allele frequencies (AF) at nuclear SSR loci. Private alleles found in accessions are in bold

BPPCT004		BPPCT008		BPPCT030		BPPCT033		pchcms5		pchgms1		pchgms2	
AS	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**
190	0.129	92	0.500	148	0.919	**142**	0.017	214	0.081	138	0.032	145	0.081
194	0.274	110	0.100	152	0.081	150	0.517	244	0.226	144	0.161	**149**	0.016
200	0.468	114	0.400			158	0.467	246	0.500	160	0.306	157	0.742
206	0.129							256	0.048	166	0.500	171	0.161
								266	0.145				

pchgms3		pchgms4		UDP96-005		UDP96-010		UDP97-402		UDP98-406	
AS	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**	**AS**	**AF**
171	0.016	150	0.371	106	0.276	83	0.276	130	0.048	87	0.258
181	0.016	176	0.629	114	0.052	99	0.293	146	0.938	91	0.339
195	0.613			120	0.466	101	0.103			101	0.065
197	0.355			126	0.172	103	0.328			105	0.339
				138	0.017						
				150	0.017						

primers combination pchcms5, pchgms1, UDP96-005, UDP96-010 and UDP98-406 allowed the unambiguous differentiation of all the cultivars studied.

The high rate of genetic heterozygosity increases the value of a group of genotypes in a breeding program ('Cortos Archena', 'Pacorros Archena' and 'Real Fino'). The low genetic heterozygosity could be associated with an inbreeding depression or an accumulation of non-favourable alleles. The range of genetic heterozygosities observed (from 0.46 to 0.96) is wider than the range obtained in apricot by other authors (from 0.24 to 0.65, Sánchez-Pérez *et al*., 2006) and the range of genetic heterozygosity obtained in peach (from 0.05 to 0.28, Martínez-Gómez *et al*., 2003). The presence of the rare alleles found in some cultivars could be because these cultivars have also been enriched with germplasm of different origin, or could be due to a mutation in the microsatellite sequence that should give rise to a new allele longer or shorter than the original one.

The dendrogram generated from UPGMA cluster analysis based on Nei genetic similarity (Nei *et al.*, 1983) shows the existence of two main clusters (Fig. 1). The upper cluster, composed mainly of cultivars from Murcia, was organised in two sub-clusters. Most of the accessions that belong to the «Clases» group appear grouped together in a sub-cluster, while the three clonal selections of 'Búlida' appear in the other sub-cluster. The cultivar 'Martinet', from Valencia, appears alone, well-separated from the rest of the cultivars of this cluster. The second, lower cluster was organised in three sub-clusters: two composed of cultivars from Murcia and the other composed of cultivars from Valencia. The cultivars from Murcia ('Pacorros Archena', 'Real Fino', 'Pelicano Archena', etc.) are traditional from the region but are not considered as «Clases». Only two «Clases» appear in the second cluster: 'Pericales' and 'Colorao Antón'. As a consequence, the clustering depicted by the dendrogram suggests the existence of a genetic pool of traditional cultivars from the regions of Valencia and Murcia (lower cluster), with a clear separation between them, and some transition cultivars such as 'Colorao Antón' and 'Mauricio'. On the other hand, clearly separated from this traditional group, another pool exists formed by «Clases» and 'Búlida' (upper cluster). The duality of grouping could reflect the previously-mentioned co-existence of two different genetic lineages in Spanish apricot cultivars, one European and one from North Africa. In this sense, the group «Clases» exhibits characters of the two lineages. The self-incompatibility and white flesh are characters from the African lineage, but they present higher chilling requirements and late ripening, as do the cultivars of European origin. The «Clases» are considered to be descendents of the cultivar 'Moniquí', in diverse combinations, and the results of the present experiment confirm this (Annexe 1). In this sense, 'Moniquí' shares at least one of the two alleles with most of them, except with 'Colorao Antón' at loci pchgms3 and UDP98-406, 'Pepito Blanco' at locus

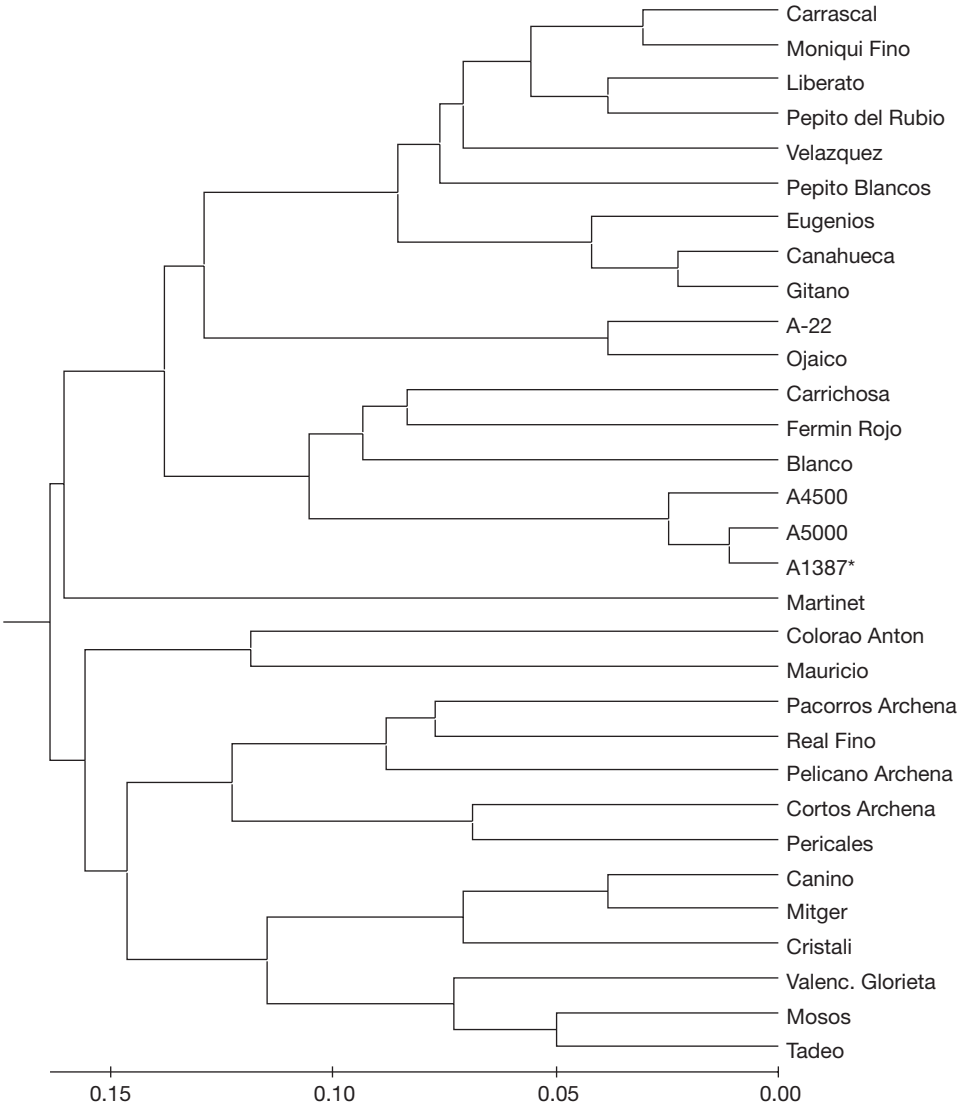

Figure 1. UPGMA dendrogram of 31 traditional Spanish apricots based on their variation at 13 SSRs loci.

pchgms3 and 'Velázquez' at locus UDP96-010. It is generally considered that 'Moniquí' could originate from France, and its presence in Murcia is considered quite ancient. This cultivar was first introduced in the Northeast of Murcia, which is colder than the Segura river basin, and is grown successfully in other areas of Spain colder than Murcia, such as Albacete and the Ebro river basin.

The clustering pattern of cultivars described here is in general agreement with previous work on the subject. Hormaza (2002) analysed, with 37 SSR primer pairs, a set of 48 apricot genotypes of diverse origin, 10 of which are coincident with ours. His data show that, within the European cultivars, there is a sub-group composed of the cultivars originating in Valencia and another sub-group composed of 'Moniquí', 'Moniquí-Borde' and two «Clases»: 'Pepito del Rubio' and 'Carrascal'. This sub-group is in turn related to other cultivars of European origin, such as 'Paviot' and 'Rouge de Rivesaltes'. The cultivar 'Búlida' seems to be closer to the Valencia group, although in the present work it seems to be transitional between the two groups. There are other papers centred on the analysis of the genetic relationships of apricot cultivars (Romero *et al*., 2003; Zhebentyayeva *et al*., 2003; Sánchez-Pérez *et al*., 2004, 2006; Krichen *et al*., 2006; Tian-Ming *et al*., 2007) but, as they analyse very different sets of cultivars, it is not possible to get a global view of the situation.

Genetic diversity and characterisation of field samples of apricot cultivar 'Búlida'

The traditional cultivar 'Búlida' occupies most of the apricot-cultivated areas in Murcia. These plants may have a dual origin: either a single seedling which produced, via vegetative propagation, different genotypes through somatic mutations or clonal selection, or more than one seedling, all with marked morphological uniformity. In order to study the potential genetic heterogeneity of clones within 'Búlida' cultivar, 66 samples identified and cultivated in fields under the 'Búlida' name, and characterised pomologically by Piñero *et al* (2006), were sampled directly in several areas of Murcia (Table 2) and analysed by testing DNA polymorphism at seven microsatellite loci (pchcms5, pchgms1, pchgms2, pchgms4, UDP96-005, UDP97-402 and UDP98-406). In addition, the 'Real Fino' variety and three clonal selections of 'Búlida' (A1387, A4500 and A5000) maintained at the IMIDA collection were included.

The resulting data for the seven loci analysed were reproducible (Annexe 2). The phylogram shows the existence of one main group of samples excluding the accession 'Real Fino', which was used as a representative outgroup in the cluster analysis (Fig. 2). Additionally, the related samples PL141, PL142 and PL143 were also placed outside of the others. The genetic similarity observed when compared to other 'Búlida' samples also identified MOL255 (genetic similarity < 0.8) as a different cultivar. Apart from these, the rest of the 65 samples showed different levels of genetic similarity - ranging between 0.89 and 0.96. The phylogram shows the existence of one main group of samples including 58 Búlida samples (from the IMIDA clonal selection A1387 to CE215) and a second group with five samples (from the IMIDA clonal selection A5000 to CA332). The third and fourth groups are represented by a single accession (CE244 and the IMIDA clonal selection A4500, respectively). No polymorphism at the DNA level was detected among the 58 samples of the main group, as well as among the five samples of the second group. These two sub-clusters were grouped at genetic similarity > 0.95, due to the existence of one polymorphism between them at the locus pchgms1 (Annexe 2). CE244 showed one and two polymorphisms, respectively, with the first and second groups of 'Búlida'. A4500 showed one polymorphism with the first group of Búlida and two polymorphisms with both the second group and CE244. Four loci had identical profiles for all the group of 'Búlidas' (pchgms2, pchgms4, pchcms5 and UDP97-402), while three showed polymorphism between them (pchgms1, UDP96-005 and UDP98-406). However, all the 65 clones grouped at genetic similarity > 0.88 showed a certain degree of genetic relatedness since they shared at least one of the two alleles. There were rare alleles detected in two 'Búlida' samples; one was only present in CE244 at locus UDP98-406 and the other in A4500 at locus UDP96-005 (Annexe 2).

The data suggest that these 65 'Búlida' samples may derive from four closely-related genotypes. Nevertheless, a possible explanation for the difference in one allele would be a somatic mutation in the microsatellite sequence of a given plant that should give rise to a new allele longer or shorter than the original one. Then, the presence of one or a few discrepancies between samples may not demonstrate that they are different. A reliable estimate of the mutation rate of the SSR used must be incorporated into the cultivar-identification procedure to make the SSR test of identity a robust and reliable one. It is interesting to remark that the genetic diversity reported here is less than the pomological diversity reported by Piñero *et al.* (2006). So, it is possible to find considerable phenotypic differences in the field that do not have a genetic base.

Conclusions

The results obtained from this study indicate that peach SSRs are useful tools for the identification and management of apricot genetic resources, and demonstrate their transportability. With respect to the genetic relationships of traditional apricot cultivars, the results corroborate the old assumption about the existence of two main genetic pools in Spain: one constituted by traditional and ancient selections in the area of Valencia-Murcia and the other, the group of «Clases», derived from this one but closer to the genetic pool of European lineage, through the connection with the cultivar 'Moniquí'. Also, the microsatellite markers offer a useful tool to characterise and identify these cultivars, whose use is now limited, but constitute a source of genetic traits of interest. In relation to the analysis of diversity of 'Búlida' in the field, this work has offered some relevant data. The phenotypic diversity found in crops in the field has prompted some authors to make clonal selections. However, the data obtained in this work indicate that the genetic diversity in the population of 'Búlida', as detected by molecular markers,

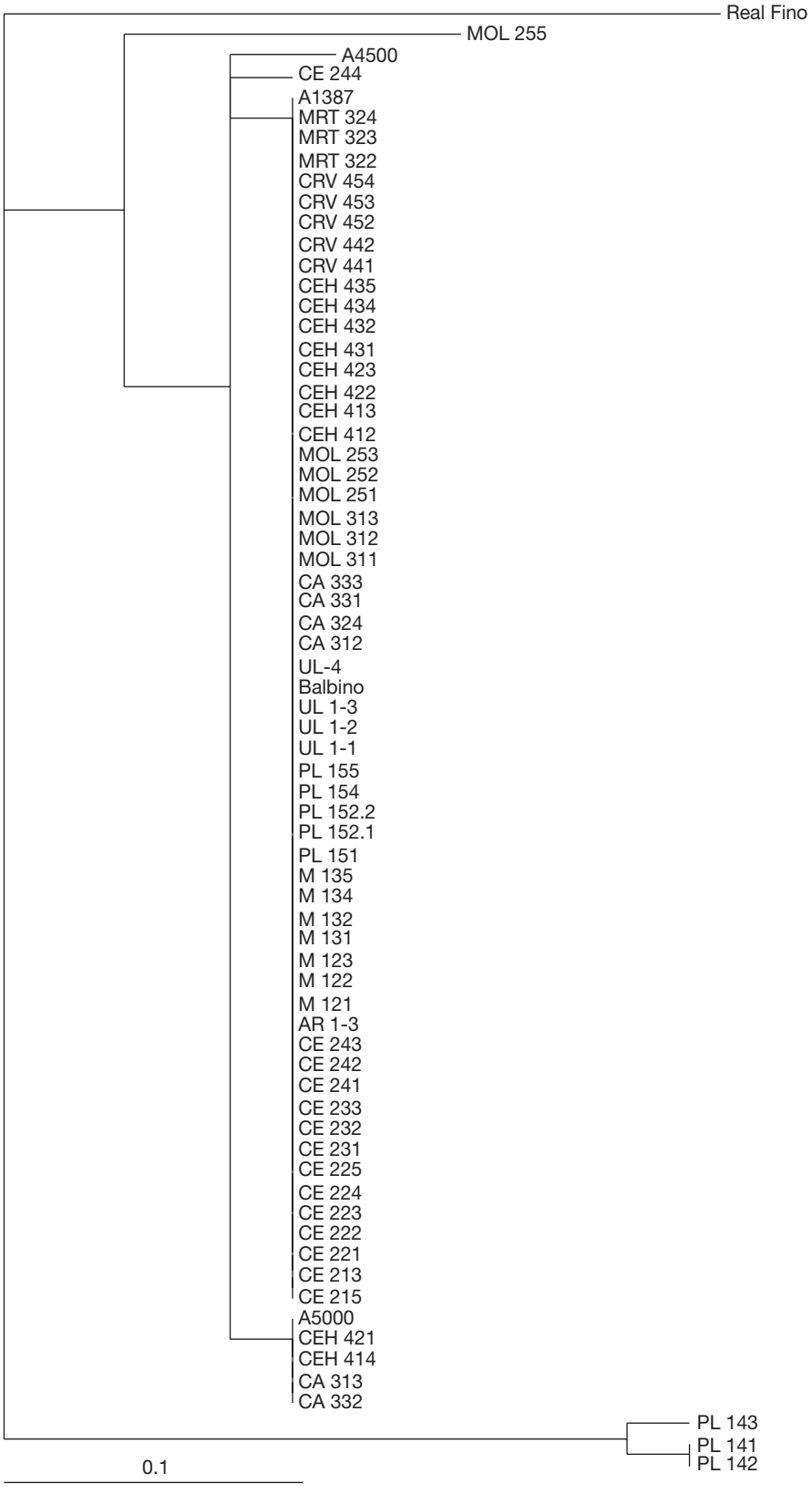

Figure 2. UPGMA phylogram of 'Búlida' samples based on their variation at seven SSRs loci.

is low. In this sense, six of the eight clonal selections of 'Búlida' obtained at the IMIDA had the same genotype. Therefore, the genotyping of candidate plants with the set of markers tested here would be useful before starting the process of clonal selection.

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Annexes

Annexe 1. Allele sizes (in bp) at 13 SSR loci for 31 traditional Spanish apricots

	pchgms1		pchgms2		pchgms3		pchgms4		pchcms5		UDP96-005		UDP96-010	
Blanco	160	166	157	157	195	195	176	176	214	246	106	150	83	83
A-22 clonal selection	144	166	157	157	195	195	150	176	246	266	106	120	101	103
Canino	160	166	157	157	197	197	150	176	214	246	106	120	83	99
Cañahueca	160	166	157	171	195	195	150	176	244	246	114	120	101	103
Carrascal	160	166	157	157	195	195	150	176	246	266	120	126	99	103
Carrichosa 2 × 5	144	166	157	171	195	195	150	176	246	246	106	120	83	83
Colorao Antón	138	160	157	157	197	197	150	176	244	246	120	126	101	103
Cortos Archena	160	166	145	157	197	197	150	150	244	246	106	120	99	103
Cristalí	160	166	149	157	197	197	150	176	246	256	106	120	99	103
Eugenios	160	166	157	157	195	195	176	176	244	266	114	120	101	103
Fermín Rojo	166	166	157	171	195	195	150	176	214	246	106	120	83	99

Annexe 1 (cont.). Allele sizes (in bp) at 13 SSR loci for 31 traditional Spanish apricots

	pchgms1		**pchgms2**		**pchgms3**		**pchgms4**		**pchcms5**		**UDP96-005**		**UDP96-010**	
Gitano	160	166	157	157	195	195	176	176	244	246	114	120	101	103
Liberato	160	166	157	157	195	195	176	176	246	256	120	126	99	103
Martinet	138	160	157	157	195	195	150	150	214	246	106	120	83	99
Mauricio	160	166	157	157	197	197	176	176	244	246	120	138	83	103
Mitjer	144	166	157	157	197	197	150	176	244	246	106	120	83	99
Moniquí Fino	160	166	157	171	195	195	150	176	246	266	120	126	99	103
Mosós	144	166	157	171	197	197	150	176	246	266	106	120	83	99
Ojaíco	144	166	157	157	195	195	150	176	244	246	106	120	101	103
Pacorros Archena	144	166	145	157	195	195	150	176	244	246	106	120	99	103
Pelícano Archena	144	166	145	157	197	197	150	176	244	246	106	120	99	103
Pepito Blancos	160	166	157	157	171	181	176	176	244	246	120	126	99	103
Pepito del Rubio	160	166	157	157	195	195	176	176	214	246	120	126	99	101
Pericales	160	160	145	157	195	195	150	150	244	246	106	120	99	103
Real Fino	144	166	145	157	197	197	150	176	246	256	106	120	99	103
Tadeo	144	166	157	171	197	197	150	176	246	266	120	126	83	99
Valenc. Glorieta	144	166	157	171	197	197	150	176	244	246	106	126	83	99
Velázquez	160	166	157	157	195	195	176	176	244	246	120	126	83	83
A5000 ‘Búlida’	160	166	157	171	195	195	176	176	246	266	120	126	83	103
A1387 ‘Búlida’	166	166	157	171	195	195	176	176	246	266	120	126	83	103
A4500 ‘Búlida’	166	166	157	171	195	195	176	176	246	266	114	120	83	103

	UDP97-402		**UDP98-406**		**BPPCT004**		**BPPCT008**		**BPPCT030**		**BPPCT033**	
Blanco	146	146	105	105	190	200	92	114	148	148	150	158
A-22 clonal selection	146	146	87	105	200	206	92	114	148	152	150	158
Canino	146	146	105	105	190	200	92	110	148	148	150	158
Cañahueca	146	146	87	91	194	200	92	114	148	152	150	158
Carrascal	146	146	87	91	194	200	92	114	148	148	150	158
Carrichosa 2 × 5	146	146	91	105	190	200	92	114	148	148	142	150
Colorao Antón	146	146	87	87	190	200	92	114	148	148	150	158
Cortos Archena	146	146	87	91	194	200	92	114	148	148	150	158
Cristalí	146	146	91	105	190	200	92	110	148	148	150	158
Eugenios	146	146	87	91	194	200	92	114	148	148	150	158
Fermín Rojo	146	146	105	105	194	200	92	114	148	148	150	158
Gitano	146	146	87	91	194	200	92	114	148	152	150	158
Liberato	146	146	87	91	194	200	92	114	148	148	150	158
Martinet	146	146	91	101	190	200	92	114	148	148	150	158
Mauricio	146	146	87	91	200	206	92	110	148	148	150	158
Mitjer	146	146	105	105	190	200	92	110	148	148	150	158
Moniquí Fino	146	146	91	105	194	200	92	114	148	148	150	158
Mosós	146	146	91	105	194	200	92	114	148	148	150	158
Ojaíco	146	146	87	101	200	206	92	114	148	152	150	158
Pacorros Archena	130	146	91	101	194	206	92	114	148	148	150	158
Pelícano Archena	130	146	91	105	194	200	92	114	148	148	150	150
Pepito Blancos	146	146	87	91	194	200	92	114	148	148	150	158
Pepito del Rubio	146	146	87	91	194	200	92	114	148	148	150	158
Pericales	146	146	87	91	194	200	92	110	148	148	150	158
Real Fino	130	146	91	101	194	206	92	110	148	148	150	158
Tadeo	146	146	87	91	194	200	92	114	148	152	150	158
Valenc. Glorieta	146	146	91	105	190	200	92	114	148	148	150	158
Velázquez	146	146	87	91	194	200	92	114	148	148	150	158
A5000 ‘Búlida’	146	146	105	105	200	206	92	114	148	148	150	158
A1387 ‘Búlida’	146	146	105	105	200	206	92	114	148	148	150	158
A4500‘ Búlida’	146	146	105	105	200	206	92	114	148	148	150	158

Annexe 2. Allele sizes (in bp) at 7 SSR loci for field samples of apricot cultivated under Búlida name

	pchgms1		**pchgms2**		**pchgms4**		**pchcms5**		**UDP96-005**		**UDP97-402**		**UDP98-406**	
CE244	166	166	157	171	176	176	246	266	120	126	146	146	103	105
A1387 'Búlida'[a]	166	166	157	171	176	176	246	266	120	126	146	146	105	105
A4500 'Búlida'	166	166	157	171	176	176	246	266	114	120	146	146	105	105
A5000 'Búlida'[b]	160	166	157	171	176	176	246	266	120	126	146	146	105	105
PL 141[c]	160	166	143	155	152	176	246	266	104	126	146	146	89	89
PL 143	160	166	143	155	152	176	246	266	104	126	136	146	89	89
MOL 255	160	166	157	157	176	176	246	266	120	126	146	146	89	93

[a,b,c] 58, 5 and 2 field samples showed these genotypes, respectively (Fig. 2).