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Genetic Diversity and Population Structure of Apricot (*Prunus armeniaca* L.) from Northern Pakistan using Simple Sequence Repeats

By M. ALI KHAN¹, F. MAGHULY², E. G. BORROTO-FERNANDEZ², A. PEDRYC³, H. KATINGER² and M. LAIMER^{2,4}

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Abstract

A collection of 44 *P. armeniaca* accessions and three related species, from diverse geographic areas, covering the entire Northern hemisphere with emphasis on Pakistani apricots, was screened with 10 SSR primer pairs developed in apricot, to characterize the cultivars and establish their genetic relationship. Given the fact that the Central Asian region is considered a center of origin of apricot, particular attention was devoted to accessions from the Hunza region of Pakistan. The primers correctly amplified a repeatable polymorphic pattern, which

unequivocally distinguished all genotypes under study. Altogether 123 alleles were identified with an average of 12.30 alleles per locus. The observed heterozygosity for individual loci ranged from 0.28 to 0.77 with an average of 0.64. A neighbour joining method identified four groups from: (A) Central Asia, (B) Irano-Caucasia, (C) Continental Europe and (D) North America. The dendrogram confirms the historic dissemination pathways of apricot from its centre of origin in Asia to the West. Apricot cultivars from the Hunza region (Northern Pakistan) revealed a high variability, as genetic diversity is still conserved due to the traditional practice of planting seeds from the best trees.

Key words: Genetic variation, microsatellites, eco-geographic groups, Hunza, center of origin.

Introduction

Botanists distinguish the following apricot species: *P. ansu*, *P. armeniaca*, *P. brigantina*, *P. mandshurica*, *P. x dasycarpa*, *P. holosericea*, *P. mume* and *P. siberica* (OECD, 2002). Most cultivated apricots, belong to the species *P. armeniaca* L., are native to Asia and Caucasus

¹Institute of Applied Biotechnology, GC University, Lahore, Pakistan.

²Plant Biotechnology Unit, Institute of Applied Microbiology BOKU, Muthgasse 18, A-1190 Vienna, Austria.

³Corvinus University, Villányi ut 29-43, 1118 Budapest, Hungary.

⁴Corresponding author: MARGIT LAIMER. University of Natural Resources and Applied Life Sciences, Institute of Applied Microbiology, Plant Biotechnology Unit, Nussdorfer Lände 11, 1190 Vienna, Austria. Tel. +43-1-36006 6560, Fax +43-1-36 97 615, Email: m.laimer@iam.boku.ac.at

and have rather small genome for a tree species ($2n=16$).

Three important regions have been described as centres of origin for cultivated apricots: the Chinese centre (China and Tibet), the Central Asian centre (from Tien-Shan to Kashmir), and the Near-Eastern centre (Iran, the Caucasus and Turkey) (VAVILOV, 1951). The Central Asian group (MEHLENBACHER et al., 1990) including Xinjiang, Afghanistan, Pakistan and Kashmir, is the oldest group with the highest genetic diversity. Geographically, the mountainous region of Northern Pakistan (34° – 36° N and 72° – 78° E) lies between three major centres of temperate fruit species diversity, the Caucasus Mountains, Central Asia and China. Apricots moved from Central Asia into the high valley “Hunza”, in the Karakorum, Himalaya, and Hindu Kush Mountain. The famous “Hunza” apricots are grown in the fertile land of Baltit or Karimabad ($36^{\circ}33'$ N and $74^{\circ}66'$ E). The small village Gulkin (2590 m above sea level) is located in the highest part of the valley, close to the Gulkin glacier. “Hunza” apricots are characterized by a long juvenile period and later blooming times, compared to European cultivars, and are commonly grown from seed. Genetic traits of greatest interest include a 3-month range in fruit ripening time, sweet pits, non-softening of fruit at maturity, very firm fruit attachment, storability of fresh fruits for several months, and very high soluble solids (to 31 deg. Brix) (LEDBETTER and PETERSON, 2004).

The Irano-Caucasian group includes apricots from Armenia, Georgia, Azerbaijan, Dagestan, Iran, Iraq, Syria, and Turkey. Most of them are self-incompatible, show lower chilling requirements and produce larger fruits than the Central Asian group. The European group, including cultivars from Europe, North America, South Africa, and Australia (MEHLENBACHER et al., 1990; LAYNE et al., 1996; FAUST et al., 1998), is the youngest and least variable group. In general, cultivars of this group show lower chilling requirements and most of them are self-fertile and precocious.

Traditional methods to characterize and identify cultivars and rootstocks of fruit tree species are based on phenotypic observations, a time consuming approach subject to environmental influences. Consequently, fingerprinting has moved towards DNA-based markers that are not influenced by the environment. Microsatellite or simple sequence repeats (SSRs) are the ideal class of genetic marker, which have many scorable and highly variable loci with co-dominant alleles, and are distributed throughout the genome.

In *Rosaceae*, SSRs first identified in apple were used in *Pyrus* to describe variation and discriminate between pear accessions (YAMAMOTO et al., 2001). Primers designed for peach SSR loci have been used to amplify loci in other Rosaceous crops (sweet and sour cherry, plum, almond, apricot, apple) and are recommended for use in almond, cherry and apricot germplasm (CANTINI et al., 2001; MARTINEZ-GOMEZ et al., 2001; HORMAZA, 2002) as well as to characterize apple genetic resources (HOKANSON et al., 1998).

In the present study, SSRs developed in apricot (LOPES et al., 2002; MESSINA et al., 2004) were used to deter-

mine genetic relationships among apricot cultivars from different geographic origin, giving special emphasis to Pakistani accessions which are valuable source of interesting traits. Hunza seedlots, representative of Central Asian apricot group, are horticulturally quite distinct from the European cultivars used in North American breeding programmes. The elevated Brix of Hunza apricots is a characteristic of major interest (LEDBETTER and PETERSON, 2004). The identification of individual accessions, among a representative set of commercially available apricot varieties, is useful information for setting any breeding strategy for apricots.

Material and Methods

Plant material and DNA extraction

Forty four apricot accessions from 14 countries representing the European, Irano-Caucasian, Central Asian and North American cultivars and one sample of *P. x dasycarpa*, *P. brigantia* and Plumcot were obtained from the germplasm collections of Corvinus University (Budapest) and BOKU University (Vienna) (Table 1). The forty-four apricot accessions include thirteen samples of Asia originated from the Northern Pakistani region of Hunza: Gulkin (1, 2, 3, 5, 6), Hunza (1, 2, 8), Karimabad (1, 2, 3, 5) and M 2000 m.

Total genomic DNA was extracted from young leaves using the DNeasy Plant Mini Kit (QIAGEN) according to the supplier's instructions.

Polymerase chain reaction (PCR) amplification and electrophoresis

All ten polymorphic primer pairs originally developed for apricot SSR loci (LOPES et al., 2002; MESSINA et al., 2004) were used for amplification of DNA from different apricot cultivars (Table 2). Optimization of annealing temperatures and $MgCl_2$ concentration for each microsatellite primer was accomplished by screening variation in annealing temperature using a BIOMETRA Gradient cyler (T-gradient).

PCR amplifications were done in 25 μ l reaction volume containing 1 \times PCR buffer (provided by the manufacturer, QIAGEN), 2 mM $MgCl_2$, 0.2 mM dNTPs, 4 pmol of each primer, 0.6 units HotStarTaq polymerase (QIAGEN HotStarTaq PCR) and 20–30 ng of total genomic DNA. Reactions were carried out in a BIOMETRA thermal cyler using the amplification profile: an initial denaturation step of $95^{\circ}C$ for 5 min, followed by 35 cycles of 50 s at $95^{\circ}C$, 50 s at annealing temperature and 1 min at $72^{\circ}C$. A final extension step of 10 min at $72^{\circ}C$ ended the cycle.

Fluorescently labelled microsatellite fragments were analysed on an ABI 3100 capillary sequencer. Fragment sizing was performed using the ABI Genotyper software.

Data analysis

For each microsatellites locus, SSR allelic composition was determined in 47 apricots. Genetic parameters like alleles per locus, effective alleles per locus (N_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) ($H_e = 1 - \sum p_i^2$, where p_i is the frequency of the i th allele),

Table 1. – List of apricot cultivars included in this study. Comp. = compatibility; SI = Self-incompatible; SC= Self-compatible; CA = Central Asian; IC = Irano-Caucasian; EE = East-European; WE = West-European; NA = North American.

Cultivar	Pedigree or cultivar group according to morphological characteristics	geographic origin	Comp.	References
Arvam aramat	-	Central Asia (CA)	-	PEDRYC et al., (pers. comm.)
Darunek malahojev	-	Central Asia (CA)	-	PEDRYC et al., (pers. comm.)
Gulkin (Five accessions)	Seedling	Pakistan (CA)	SI?	FLIRI (pers. comm.)
Hunza (Three accessions)	Seedling	Pakistan (CA)	SI?	FLIRI (pers. comm.)
Karimabad (Four accessions)	Seedling	Pakistan (CA)	SI?	FLIRI (pers. comm.)
Khurmai	-	Central Asia (CA)	SI	LÖSCHING et al., 1954
M2000 m	Seedling	Pakistan (CA)	-	FLIRI (pers. comm.)
Samarkandskij rannij	Krasznoscokij × Majszkaja szkoroszpelka	Uzbekistan (CA)	SI	BROZIK et al., 2000
Zard 2407	-	Central Asia (CA)	SI	PEDRYC et al., (pers. comm.)
Erevan (syn.: Shalakh)	Local variety in Armenia	Armenia, Irano-Caucasian (IC)	SC	Lösching et al., 1954; ROMERO et al., 2003
Harmat	Seedling from open pollinated Jubilar (Jubilar is an open pollinated seedling of Shalakh)	IC	SI	ROMERO et al., 2003
Junskij	Shalakh open pollinated	Moldavia (IC)	-	PEDRYC et al. (pers. comm.)
Korai zamatos	Jubilar (Shalakh) open pollinated	IC	SI	BROZIK et al., 2000
Voski 2425	Sateni open pollinated	Armenia (IC)	SI	PEDRYC et al., (pers. comm.)
Bahrt (Orangered)	Lasgerdi Mashhadi × NJA2	USA (NA)	SI	LICHOU, 1998
Goldrich	Sungold × Perfection	North America (NA)	SI	LICHOU, 1998
Harcot	[Geneva × Naramata) × (Morden 604) × (Phelps × Perfection)]	Canada 1968 (NA)	SI	HAGEN et al., 2002; LICHOU, 1998; ROMERO et al., 2003
Plumcot	<i>P. armeniaca</i> × <i>P. salicina</i>	USA (NA)	SC	http://WWW.digitalseed.com/gardener/fruit/apricotplum.html
Colonel	-	East Europe (EE)	-	PEDRYC et al., (pers. comm.)
Comandor (syn: Marculesti 18/6)	Marculesti 17/52 (Luizet × Umberto) × Marculesti 43/1 (Silistra × Ananas)	Romania, 1985 (EE)	SC	PEDRYC et al., (pers. comm.)
Klosterneuburger Marille	-	East Europe (EE)	-	MODI (pers. comm.)
Kosztjuzszuskij	-	East Europe (EE)	-	PEDRYC et al., (pers. comm.) ZIEBENTYAYEVA et al., 2003
Krimskij amur	Mulla sadik × Udarnik	Ukraine (EE)	SC	and PEDRYC et al., (pers. comm.)

Table 1. – (Continued).

Cultivar	Pedigree or cultivar group according to morphological characteristics	geographic origin	Comp.	References
Mamaia	-	East Europe (EE)	-	PEDRYC et al., (pers. comm.)
Medunec	-	East Europe (EE)	-	PEDRYC et al., (pers. comm.)
Nagyezsda	-	East Europe (EE)	-	PEDRYC et al., (pers. comm.)
Narjadnij	Oranshevo Krasnij × Shirazkij	Ukraine (EE)	-	PEDRYC et al., (pers. comm.)
Pasinok 1464	Vinoslivij × Erevan	Ukraine (EE)	SC	PEDRYC et al., (pers. comm.)
Priusadebnij	Samarkandskij rannij × Krasnoshchokij	Ukraine (EE)	SI	PEDRYC et al., (pers. comm.)
Rosensteiner	Seedling	East Europe (EE)	SC	MODL (pers. comm.)
Rózsa C.1406	"Rose " group	Hungary 1955 (EE)	SC	BROZIK et al., 2000
Bergeron	Unknown seedling , Lyon , France	France, Lyon 1920 (WE)	SC	BROZIK et al. 2000; LICHOU, 1998; LICHOU et al., 2003
Moniqui	-	Spain (WE)	SI	LICHOU, 1998
<i>P. x dasycarpa</i> Ehrh. (black apricot)	<i>P. cerasifera</i> × <i>P. armeniaca</i>	Italy (WE)	-	OECD, 2002
<i>Prunus brigantia</i> Vill.	<i>P. brigantia</i> (Alpine plum)	France 1779 (WE)	SC	JACOBSON, 2003 (web site)
Rouge de Sernhac	Chance seedling, Sernhac, France	France, 1973 (WE)	SC	LICHOU, 1998
Vinschger Marille	Old local variety	Italy (WE)	SC	STRADA et al., 1989

pers. comm = personal communication.

"-" = No information.

Table 2. – Characteristics of the 10 SSRs loci isolated from apricot, used in this study.

Locus	predicted length (bp)	Size range (bp)	Annealing temperature	Repeat motife	Reference
ssrPaCITA7	211	186-222	60°C	(AG) _n	Lopes et al. 2002
ssrPaCITA10	175	144-188	58°C	(CT) _n	Lopes et al. 2002
ssrPaCITA19	114	98-148	60°C	(TC) _n	Lopes et al. 2002
ssrPaCITA23	146	112-153	56°C	(AC) _n (AG) _n	Lopes et al. 2002
ssrPaCITA27	262	226-264	58°C	(TC) _n (TA) _n (TG) _n	Lopes et al. 2002
UDAp-407	188	118-162	58°C	(TC) _n TT(TC) _n	Messina et al. 2004
UDAp-410	155	116-154	58°C	(AG) _n	Messina et al. 2004
UDAp-414	174	150-214	58°C	(AG) _n	Messina et al. 2004
UDAp-415	156	139-143	58°C	(GA) _n	Messina et al. 2004
UDAp-420	175	154-242	58°C	(CT) _n	Messina et al. 2004

among population component of the inbreeding coefficient (F_{ST}) were measured as described by LEVENE, 1949 and NEI, 1973 and were calculated using POPGENE (version 1.32; YEH and BOYLE, 1997). A neighbor-joining

tree (NJ-Tree; SAITOU and NEI, 1987) was generated with the 47 individuals according to NEI's (1972) standard genetic distance, using Population 1.2.28 (LANGELLA, 2000) and TreeView 1.6.6 (PAGE, 1996).

Table 3. – Number of observed allele (na), Effective alleles per locus (ne), observed (H_o) and expected (H_e) heterozygosity and F_{ST} calculated for 10 SSR markers in 47 apricot cultivars.

Locus	na	ne	H_o	H_e	F_{ST}
ssrPaCITA7	13	7.26	0.77	0.87	0.55
ssrPaCITA10	14	6.25	0.58	0.85	0.65
ssrPaCITA19	10	5.43	0.77	0.82	0.53
ssrPaCITA23	8	4.23	0.63	0.77	0.59
ssrPaCITA27	6	3.66	0.28	0.73	0.82
UDAp-407	21	10.43	0.71	0.91	0.61
UDAp-410	14	6.50	0.75	0.86	0.56
UDAp-414	16	6.25	0.63	0.85	0.63
UDAp-415	12	3.31	0.60	0.71	0.57
UDAp-420	9	4.72	0.68	0.80	0.58
Mean	12.30	5.80	0.64	0.82	0.61
St.Dev	4.35	2.08	0.14	0.06	-

Results

Microsatellites diversity

Amplified microsatellites loci were scored in 47 *Prunus* accessions. Polymorphic bands were obtained with all primers. Alleles were clearly differentiated using the capillary electrophoresis sequencer. The number of alleles detected among the 10 loci studied ranged from 6 to 21 alleles per locus (Table 3). A total of 123 alleles were detected with a mean number of 12.3 alleles per locus.

Locus UDAp-407 was the most polymorphic among the ten loci (21 alleles), with the highest effective number of alleles (Table 3), while locus ssrPaCITA27 was the least polymorphic (six alleles). The observed heterozygosity, calculated for each individual loci, ranged from 0.28 in locus ssrPaCITA27 to 0.77 in locus ssrPaCITA 7 and locus ssrPaCITA 19, with an average of 0.64 (Table 3). In all individuals, the observed heterozygosity (H_o) was lower than the expected heterozygosity (H_e), which can result from an intensive selection process.

Out of the 123 different alleles detected in all individuals, 37 single alleles were amplified with 7 loci as follow: At locus ssrPaCITA 10 (Arvam armut, Colonel, Gulkin 6, Harcot, Karimabad 5, Shalakh), at locus ssrPaCITA 19 (Narjadnij, Rouge de Sernhac), at locus ssrPaCITA 23 (Junskej, Harcot), at locus UDAp-407 (Erevan, Junskej, Priusadebnij), at locus UDAp-410 (Arvam armut, Gulkin 1, Harcot, Plumcot), at locus UDAp-414 (Bahrt, Gulkin 3, Harcot, Hunza 1, Plumcot, *P. brigantina*, Voszki), at locus UDAp-415 (Churmai, Colonel, Karimabad 6, *P. brigantina*, Vinschger Marille) (Table 3).

Non-amplifying (null) alleles were observed in two loci (ssrPaCITA 27, UDAp-420) of three accessions (Zard, *P. brigantina* and Darunek malahojev). F_{ST} values ranged from 0.53 to 0.82 (ssrPaCITA 27), with an average of 0.61 (Table 3). According to WRIGHT (1951), the high F_{ST} values (>0.25) describe great genetic differenti-

Table 4. – Mean number of observed allele (na), Effective alleles per locus (ne) and F_{ST} across the 10 microsatellites loci in four different eco-geographic regions.

Geographic origin	Mean of na	Mean of ne	Mean of F_{ST}
Irano Caucasian	4.7	3.57	0.45
European	5.5	3.31	0.56
Central Asian	7.9	4.31	0.59
North American	4.0	3.24	0.44

ation (HARTL and CLARK, 1997), and the low gene flow, which could reflect different geographic origins or human impact.

Among different geographical origins analysed with the 10 SSR markers, the highest mean number of observed alleles and the level of expected heterozygosity was detected in the Central Asian group (Table 4).

Genetic relationships among cultivars

A neighbour-joining tree was built to classify all individuals (Figure 1). The tree divided the accessions into five groups (A, B1, B2, C, and D). Group A contains only cultivars from Central Asia (15 accessions). All Irano-Caucasian cultivars (8 accessions) cluster in two groups (B1 and B2). The apricot cultivars from Group C originate from Eastern and Western Europe. The American apricot group D includes Bahrt, Orangered, Harcot and Goldrich, which have European and Asian ancestors (Figure 1 and Table 1). The dendrogram reflect the pedigree of the cultivars. *P. x dasycarpa* and Plumcot, which are hybrids of *P. armeniaca*, were found intermediate between the apricot cultivars.

Discussion

According to VAVILOV (1951) the centres of apricot domestication are located in Asia. KOSTINA (1946) sug-

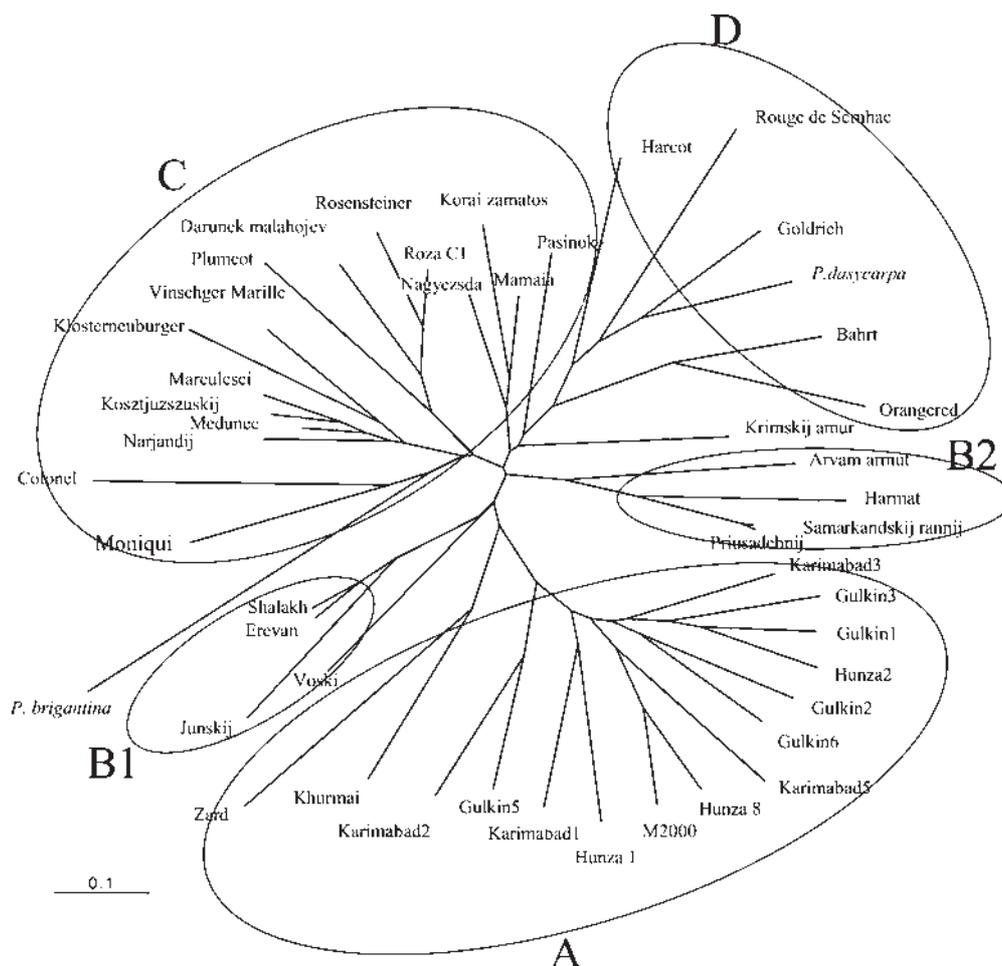


Figure 1. – Neighbour-joining (NJ) dendrogram of 47 apricot accessions with 10 apricot SSRs loci. Geographical origins are shown as (A) Central Asian, (B) Irano-Caucasian, (C) European and (D) North American.

gested that apricots were disseminated from Central Asia westwards through Iran and the Transcaucasian area. In Central Asia, native cultivars were most likely derived from wild *P. armeniaca*, and co-cultivation of wild and domesticated forms was practiced in the mountainous area from the Kazakhstan-Chinese border, south to Kashmir and west into Afghanistan. Apricots were introduced into the Northern area of Pakistan from Central Asia (THOMPSON, 1998). A secondary Near-Eastern Centre of domestication is the mountainous region from north eastern Iran to the Caucasus Mountain and central Turkey (VAILOV, 1951).

The grouping of Northern Pakistan apricot with those from Central Asia (like Khurmai and Zard) and neighbouring with those from Irano-Caucasus in the “B” group of neighbour-joining tree in our study supports this hypothesis and reflects their geographical origins and domestication history. The group including 13 seedlings from Pakistan showed a high variability of different alleles (Table 4), reflecting the Pakistani practice of planting seeds from the best trees over an extending period of time, which resulted in a high amount of variation and conservation of genetic traits (THOMPSON, 1993; HAGEN et al., 2002).

Most of the apricot breeding programmes in Europe were based on the use of local cultivars (AUDERGON, 1995). In the past, breeding programmes in North America generally were concentrated by hybridization involving only the European eco-geographical group (BAILEY and HOUGH, 1975), because world politics limited the access to Asian Centers of origin of temperate fruit trees (THOMPSON, 1993). Near the end of the 1980s, western fruit explorers began gaining access to numerous regions, previously unavailable to them, nicely illustrated by the cultivar Bahrt and Orangered, a hybrid of Lasgerdi Mashadi (Irano-caucasian) x NJA2 in the North American cluster. Thus, the introduction of apricot in North America followed at least two routes, one Asian and the other European germplasm (KOSTINA, 1969; BAILEY and HOUGH, 1975; BADENES et al., 1996; FAUST et al., 1998). This increased their level of polymorphism compared with the European cultivars.

The results of the neighbour-joining tree analysis placed all European accession in one group “C”, although within this group, the Spanish genotype e.g. Moniqui, has some differential traits such as the presence of some self-incompatible genotype, lower chilling requirement, earlier blooming and fruit maturity. Given

these characteristics, the Spanish apricot germplasm was proposed by EGEA et al. (1988) to have arisen by the confluence of North African genotypes brought by the Arabs. The remaining European apricot cultivars most likely originated from a few forms brought from the near-Eastern region and this is supported by the grouping of several European cultivars with those from Irano-Caucasian and Central Asian germplasm. The presence of the cultivars Narjandij [hybrid of Oransherokrasnij x Shirazkij (Irano-caucasian)], Pasinok [hybrid of (Erevan, Irano-caucasian) x Vinoslivij], Koraizamatos (Shalakh, Irano-caucasian) within European group corroborate this hypothesis. European cultivars have the same SSR allelic profile as Irano-Caucasian and widespread Central Asian cultivars, which reflects not only the above mentioned strategy to enlarge the genetic variability of European cultivars, but also support the hypothesis of dissemination of apricot from Central Asia westwards through the Irano-Caucasian region into Europe. Through the neighbour-joining tree, the cluster "D", found in the vicinity of Irano-Caucasian accessions, comprises of North American cultivars and Western European cultivars.

P. armeniaca hybrids (*P. x dasycarpa* and plumcot) were placed between the apricot cultivars. *P. x dasycarpa*, called the black apricot, is an apricot/plum hybrid found in the wild or produced through controlled crosses. Plumcot is another hybrid of apricot with *P. salicina*. Both accessions may become increasingly useful in plant breeding schemes to introgress unique agronomic traits into common apricots or rootstocks.

In the neighbour-joining tree all the related species were placed far from the common apricot cluster. The most remote from the common apricot is *P. brigantia* or the Alpine Plum, which is found in the Alps near Briançon (France). It has several morphological differences from the common apricot, such as its prune-like fruit, dark bark and inflorescence type. TAKEDA et al. (1998) and MAGHULY et al. (2005, 2006), using SSR and RAPD (RUTHNER et al., 2003) markers, also found *P. brigantia* at a remote position from commercial apricot cultivars, which is concordance with our results.

The obtained average number of observed alleles per locus was 12.30, which was higher than 4.1 detected by HORMAZA (2002) with 19 polymorphic SSRs in 48 different apricot genotypes, the 7.64 noticed by ZHEBENTYAYEVA et al. (2003) in 74 native apricot accessions with 14 SSRs and 3.1 observed by ROMERO et al. (2003) in a set of 40 apricots cultivars with 16 microsatellites. However, the value is comparable with 13.30 detected by MAGHULY et al. (2005) in 133 apricot cultivars with the same set of SSRs markers.

In our set of apricot cultivars, SSR markers detected a considerable polymorphism and were efficiently used for fingerprinting purposes. High allele number and heterozygosity reflect the ability of SSR markers, to provide unique genetic profiles for individual plant genotype.

The high diversity of apricots available in Central Asia, particularly in Northern Pakistan, should be included in a responsible manner into breeding programmes.

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