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Simple sequence repeat (SSR) analysis for assessment of genetic variability in apricot germplasm

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Abstract Thirty SSR primer combinations, developed from peach SSR-enriched genomic libraries and BAC libraries of peach [*Prunus persica* (L.) Batsch.], were tested for cross amplification with 74 apricot (*Prunus armeniaca* L.) germplasm accessions. Twelve primer pairs amplified 14 polymorphic SSR loci useful for discriminating most apricot cultivars, as well as for investigating patterns of variation in apricot germplasm. Levels of polymorphism were higher than the levels described using other codominant marker systems (i.e., isozymes, RFLP markers). Overall, 107 alleles were identified, and all but 11 accessions were unambiguously discriminated. Genetic differentiation of native germplasm into traditional ecogeographical groups was low, with a high level of genetic identity (> 0.75) between the groups. However, neighbor joining cluster analysis of marker distances between cultivars reflected the complex history of apricot domestication, producing groupings not evidently based on the geographical origin of the cultivars. Distant positioning of Chinese cultivars on UPGMA and neighbor joining dendrograms supports the authors' consideration of Chinese apricots as subspecies, *Prunus armeniaca* var.*ansu* Maxim., rather than a separate species.

Keywords Genetic variation · Genetic resources · Microsatellite · Crop domestication · *Prunus armeniaca*

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Introduction

Apricot is an economically important member of the Rosaceae, in the genus *Prunus* L., subgenus *Prunophora* Focke and section *Armeniaca* (Lam.) Koch (Rehder 1967). Depending on the classification system, the number of apricot species ranges from three to ten. Four distinct species are most commonly recognized: *Prunus armeniaca* L., *Prunus mandshurica* (Maxim.) Koehne, *Prunus sibirica* L. and *Prunus mume* (Sieb.) Sieb. & Zucc. All apricot species that have been studied are regular diploids, with eight pairs of chromosomes (2n = 16), and all can be intercrossed in either direction (Mehlenbacher et al. 1990).

On the basis of morphological characters and pomological descriptions, most cultivated apricots have been attributed to one species, *P. armeniaca*. Four major ecogeographical groups (Central Asian, Irano-Caucasian, European, Dzungar-Zailij) with regional subgroups have been described, according to their ecological adaptation (Kostina 1936, 1964). Recently, North and East Chinese groups were added to this classification (Layne et al. 1996). Between the groups, apricots have characteristic differences in the predominant types of trees and fruit, as well as in important physiological traits such as winter dormancy period, self-compatibility and disease resistance. A number of intermediate forms are described within most of the groups and subgroups.

During the past few decades, isozyme markers have been used to assess genetic variability in apricots (Byrne and Littleton 1989; Battistini and Sansavini 1991; Badenes et al. 1996). However, isozyme analysis does not detect enough polymorphism to discriminate all cultivars and reveal relationships among them. Only North American cultivars, with putative introgression of Central Asian germplasm, have been clearly distinguished from European and Irano-Caucasian cultivars (Badenes et al. 1996).

The availability of DNA-based markers provides a new possibility for evaluating biodiversity among plant genomes (Karp et al. 1996). RFLP markers developed for European and North American apricots produced

unique profiles for almost all cultivars (De Vicente et al. 1998). Spanish cultivars were clustered together and were distant from the remaining European and North American forms. RAPD markers were also employed to assess genetic variability of apricot germplasm of different origin (Takeda et al. 1998; Zhebentyayeva and Sivolap 2000). However, RFLP markers did not display sufficient polymorphism to analyze related germpasm, and reproducibility of RAPD markers is sometimes problematic.

Simple sequence repeats (SSRs or microsatellites) have become the genetic markers of choice in many plant species because they are PCR-based, highly reproducible, polymorphic, generally codominant and abundant in plant genomes (Powell et al. 1996). In many cases, DNA sequences flanking SSRs are conserved between taxa. Transportability of SSR markers across species in the same genus has been observed in *Vitis* (Di Gaspero et al. 2000) and *Brassica* (Plieske and Struss 2001). In Rosaceae, SSRs first identified in apple were used in *Pyrus* to discriminate between pear accessions and describe variation among them (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 comparative mapping within the family (Cipriani et al. 1999; Sosinski et al. 2000). SSRs have been used to assess variation in almond, cherry and apricot germplasm (Cantini et al. 2001; Martinez-Gomez et al. 2001; Hormaza 2002), as well as characterize apple genetic resources (Hokanson et al. 1998).

One of the world's largest collection of apricots is maintained at Nikita Botanical Garden (Crimea, Ukraine) and contains >700 accessions representing all ecogeographical groups and subgroups of cultivated apricot, wild forms of *P. armeniaca,* and all related species of section *Armeniaca*. The accessions reflect almost all biodiversity of the crop (Kostina 1931; 1936; Kovaljov 1963). Utilization of SSR markers as anchors in different *Prunus* genetic maps can facilitate efforts to search within aborigine apricot germplasm for donors of agronomically important genes.

In the present study, we use peach SSR primer pairs for cross-species amplification in apricot in order to: (1) examine patterns of SSR polymorphism among cultivars and determine whether the cultivars have unique SSR profiles; (2) investigate genetic relationships among native cultivated apricots that originated under the influence of different ecological factors and ethnic traditions of cultivation; and (3) estimate the extent of variation in apricot germplasm, between and within ecogeographical groups and subgroups.

Materials and methods

Plant material

Seventy four native apricot accessions were chosen to represent the European (15), Irano-Caucasian (10), Chinese (11) and Central Asian (32) ecogeographical groups of cultivated apricot, as well as six Dzhungar-Zailij forms from wild population of *P. armeniaca* (Table 1). The Central Asian cultivars belonged to Fergana (13), Khorezm (3), Zeravshan (9) and Kopet-Dag (7) subgroups. To avoid sampling recently introduced cultivars, we included only accessions that reportedly originated in the respective regions. To verify allelic composition of the SSR loci, nine hybrid cultivars of known pedigree were also evaluated.

DNA extraction

Total genomic DNA was extracted using the modified CTAB procedure described by Sivolap et al. (1998). Fresh young leaves (100–150 mg) were ground in 0.5 ml of extraction buffer (100 mM Tris-HCl, pH 8.0; 1.4 M NaCl; 20 mM EDTA; 5 mM dithiothreitol; 2% SDS). The samples were incubated for 60 min at 37 °C in a water bath, protein was removed by extraction with chloroform-isopentanol (24:1) two times, and DNA was precipitated with isopropanol (1:1) and washed with 70% ethanol. The pellet was dissolved in 300 µl of 1 × TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA), treated with RNAse A (1 µg/ml, 60 min, 37 °C) and proteinase K (100 µg/ml, 30 min, 37 °C). Ammonium acetate (8 M) was added to a final concentration of 2 M, and nucleic acids were precipitated by the addition of 2 v/v of ethanol and redissolved in 0.5 ml 1 × TE buffer. Stock solutions of DNA were stored at –20 °C. DNA concentrations were measured using a fluorometer (Hoefer Scientific, San Francisco, Calif.). Working solutions of genomic DNA at 10 ng/µl in 0.1 × TE buffer were prepared for SSR analysis and kept at 4 °C.

PCR amplification and electrophoresis

Thirty primer combinations, originally developed for peach SSR loci and representing different regions of the peach genome, were used for amplification of cultivated apricot DNA (see Table 2). Each PCR reaction (10 µl final volume) contained 5 ng of genomic DNA, 1 × PCR buffer (10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM $MgCl_2$), 200 µM of each dNTP, 0.12 µM of each primer and 0.5 U of Ampli *Taq* DNA polymerase (PE Biosystems). The forward primer was 5′ end-labeled with γ^{33}P-ATP using T4 polynucleotide kinase (Promega). PCR reactions were performed in an MJ PT-200 thermocycler (MJ Research Inc., Waltham, Mass.). The amplification profile consisted of an initial denaturation for 4 min at 94 °C followed by 32 cycles of 45 s at 94 °C, 30 s at the annealing temperature (40–60.5 °C, depending on primer design), 30 s elongation at 72 °C, and a final extension step of 5 min at 72 °C. The reaction products were denatured by heating for 3 min at 94 °C with equal volume of tracking dye (98% formamide, 10 mM of EDTA, 0.25% each of bromphenol blue and xylene cyanol), then electrophoresed on 6% denaturing gels (19:1 acrylamide-bisacrylamide, 7.5 M urea) in 1 × TBE buffer (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8.0) at 70 W for 2.5–6 h, depending on the fragment sizes. Each gel included lanes of the molecular size marker pGEM –3zf(+) (Promega), which was labelled with γ^{33}P-ATP and denatured per manufacturer's instructions. After electrophoresis, gels were transferred to filter paper, dried and exposed to X-ray film (Biomax MR, Kodak) for 3–5 days.

Data analysis

For each of the defined loci, SSR allelic composition was determined for each accession. Putative alleles were indicated in alphabetical order (A for smallest fragments, etc.). The program POPGENE version 1.31 (Yang and Yeh 1993) was used to calculate allele frequencies, alleles per locus (N_e), observed heterozygosity (H_o) and Nei's (1973) expected heterozygosity (H_e) for each locus. Nei's (1978) genetic identity **I** and genetic distance **D** were calculated for all pairs of ecogeographical groups and subgroups (except Khorezm), and values of **D** were used to conduct

Table 1 Apricot cultivars included in this study

European	*Chinese*	*Central Asian/Kopet-Dag*
Ananasnyi Tsurupinskij	Da-chuan-che N1	Lyuchak Sumbarskii
Velkopavlovička	Da-chuan-che N2	KB-12
Vengerskii Krupnyi	Yuan-sin	B-1-11
Krasnoshchekii	Kitaiskii	KB-9
Yubilejnyi	Shantungskii	B-4-5
Alberge de Tur	Da-bei	B-5-3
Tilton	Mai-che-sin	KK(2) N1
Luizet Krupnoplodnyi	In-ben-sin	
Kantsler	Lao-yech-lian	*Central Asian/Zeravshan*
Early Gold	Pui-sha-sin	Arzami
De Compot	Mi-Bada	Gulyunghi
Precoce d'Italia		Iskadari
Real d'Imola	*Central Asian/Fergana*	Rukhi Dzhuvanon Meona
Vynoslyvyi	Samyi Rannii	Rukhi Dzhuvanon Surkh
Bergeron	Kok-Pshar	Maftobi
	Supkhani	Badami
Irano-caucasian	Kandak-10	Kunduzi
Shalakh	Kandak-12	Mamuri
Vaagas Vardaguin	Khurmai	
Ordubad	Khurmai Rannii	*Dzhungar-Zailij*
Katuni	Kzyl Khurmai Kannibadam	Dzhungarskii 18/55
Nasera Tabriz	Oranzhevo-krasnyi	Dzhungarskii 18/63
Kurbane Marache	Mirsandzhali	Dzhungarskii 18/64
Daradzhi ek Shabistr	Kolon Boboi	Dzhungarskii 18/68
Shakarpara de Semnan	Kzyl Uryuk	Dzhungarskii 18/71
Mascat	Tadzhabai	Dzhungarskii 18/75
Nakhichevanskii		
	Central Asian/Khorezm	*Hybrid*
	Nukul Citronnii	Krimskii Amur (Mulla Sadik × Udarnik)
	Kzyl Khorezmskii	Lunnik (Vynoslivyi × Shalakh)
	Paivandi Bukharskii	Shedevr (γ-irradiated mutant of Lunnik)
		Olimp (Vynoslivyi × Shalakh)
		Parnas(Vynoslivyi × Shalakh)
		Naslazhdenije(Vynoslivyi × Shalakh)
		Dionis (Shalakh × Samarkandskii Rannii*)
		Medunets Krimskii [Oranzhevo-krasnyi × Krasnoshchekii) × Shalakh]
		Priusadebnyi (Krasnoshchekii × Samarkandskii Rannii)

* Samarkandskij Rannii is believed to be an offspring of Samyji Rannii

cluster analysis with a UPGMA algorithm and construct a dendrogram. Pairwise genetic distances among the 74 accessions were calculated (as 1-proportion of shared alleles), using the program MICROSAT (Minch 1997); cluster analysis was conducted with a neighbor joining algorithm, using the NEIGHBOR program in PHYLIP version 3.5c (Felsenstein 1989); and a dendrogram was constructed, using the program TREEVIEW (Page 1996). For both cluster analyses, the analysis was conducted ten times, with samples in randomized order each time, and the ten analyses were compared using the CONSENSE program in PHYLIP.

Results

Cross-species amplification of apricot DNA with peach SSR primers

Eighteen of the 30 peach SSR primer combinations (60%) produced prominent and strong PCR products (Table 2). For 16 putative loci, the size range of amplified fragments was approximately the same as in peach. Two primer pairs (pchgms 17 and pchgms 18) produced DNA fragments >150 bp longer than the corresponding SSR marker fragments in peach. Three primer pairs (pchgms 5, pchgms 10 and pchgms 26 F2, R2) yielded complex banding patterns, suggesting that multiple loci were amplified using a single primer pair. The 18 primer pairs had different levels of fragment size variation. Six pairs failed to reveal any variation in the accessions tested and 12 pairs amplified polymorphic markers. Of these, two pairs (pchgms 10 and pchgms 26 F2, R2) each amplified polymorphic markers in two fragment size zones, and pchcms 5 amplified one polymorphic marker and one monomorphic band.

Segregation characteristics at each putative SSR locus were determined by comparing allelic composition of hybrid cultivars and their parents. For all of the polymorphic loci except three, alleles appeared to be inherited in a Mendelian manner, and allelic composition in the hybrid cultivars was predictable from paternal and maternal genotypes (Fig. 1). In the cultivars Vynoslivyi and Shalakh and their hybrids (Table 1), nonamplifying alleles were observed at two polymorphic loci (pchgms 10-2 and pchgms-26 F2, R2-2) and monomorphic locus

Table 2 Peach-apricot transportability of 30 SSR markers revealed by amplification of peach designed primers with apricot DNA

Primer	Amplification of apricot DNA	Product size (bp)	Polymorphic Yes/No	Sequence reference
pchcms 1	No PCR product (very faint)			Sosinski et al. (2000)
pchgms 3	Distinct PCR product	171–202 (179)*	Yes	Sosinski et al. (2000)
pchgms 4	Distinct PCR product	155 (174)	No	Sosinski et al. (2000)
pchcms 5	Complex band pattern (1 scored zone)	[1]248–310 (246) [2]242	Yes No**	Sosinski et al. (2000)
pchgms 7	No PCR product			Rajapakse et al. (2001)
pchgms 10	Complex band pattern (2 scored zones)	[1]183–186 [2]198–202 (198)	Yes Yes	Wang et al. (2002
pchgms 11 F1, R1	Distinct PCR product	199–201 (229)	Yes	Wang et al. (2002
pchgms 11 F2, R2	Distinct PCR product	267 (286)	No	Wang et al. (2002
pchgms 12	Distinct PCR product	448–490 (433)	Yes	Wang et al. (2002
pchgms 14	Distinct PCR product	~520–550 (500)	Yes	Wang et al. (2002
pchgms 15	No PCR product			Wang et al. (2002
pchgms 16	No PCR product			Wang et al. (2002
pchgms 17	Distinct PCR product	463–465 (219)	Yes	Wang et al. (2002
pchgms 18	Distinct PCR product	~600 (419)	No	Wang et al. (2002
pchgms 19	No PCR product			Wang et al. (2002
pchgms 20 F1, R1	Distinct PCR product	249–267 (252)	Yes	Wang et al. (2002
pchgms 20 F2, R1	Distinct PCR product	~540–580 (560)	Yes	Wang et al. (2002
pchgms 21 F1, R1	Distinct PCR product	274 (288)	No	Wang et al. (2002
pchgms 21 F1, R2	Distinct PCR product	234 (250)	No	Wang et al. (2002
pchgms 22	No PCR product			Wang et al. (2002
pchgms 23	No PCR product			Wang et al. (2002
pchgms 24	Distinct PCR product	505 (509)	No	Wang et al. (2002
pchgms 25	No PCR product			Wang et al. (2002
pchgms 26 F2, R2	Complex band pattern (2 scored zones)	[1]372–378 (341) [2]245–319	Yes Yes	Wang et al. (2002
pchgms 26 F1, R1	No PCR product			Wang et al. (2002
pchgms 30	No PCR product			L. Georgi, personal communication
pchgms 31	No PCR product			L. Georgi, personal communication
pchgms 32	No PCR product			L. Georgi, personal communication
UPD 96-001	Distinct PCR product	111–113 (120)	Yes	Cipriani et al. (1999)
UPD 98-46	Distinct PCR product	86–114 (101)	Yes	Cipriani et al. (1999)

* Size of peach SSR fragments
** Putative dominant inheritance

Table 3 Characterization of polymorphic SSR loci in apricot

Locus	Number of putative alleles	Effective alleles per locus (Ne)	Observed heterozygosity (Ho)	Expected heterozygosity (He)
pchgms 3	11(A–K)*	6.62	0.838	0.849
pchcms 5-1	16(A–R)	6.25	0.784	0.840
pchgms 10-1	3(A–C)	2.45	0.392	0.592
pchgms 10-2	6(A–F)	3.89	0.324	0.849
pchgms 11 F1, R1	3(A–C)	1.46	0.311	0.316
pchgms 12	11(A–K)	8.02	0.743	0.875
pchgms 14	6(A–F)	3.14	0.594	0.682
pchgms 17	2(A, B)	1.22	0.176	0.182
pchgms 20 F1, R1	14(A–N)	7.69	0.824	0.870
pchgms 20 F1, R2	7(A–G)	2.79	0.446	0.642
pchgms 26 F2, R2 –1	3(A–C)	1.75	0.378	0.430
pchgms 26 F2, R2 –2	10(A–J)	4.37	0.216	0.771
UPD 96-001	2(A,B)	1.60	0.473	0.375
UPD 98-406	13(A–M)	6.94	0.743	0.856
Mean	7.64	4.16	0.517	0.645
St. dev	4.81	2.48	0.233	0.232

* Letters in parentheses refer to allele designation

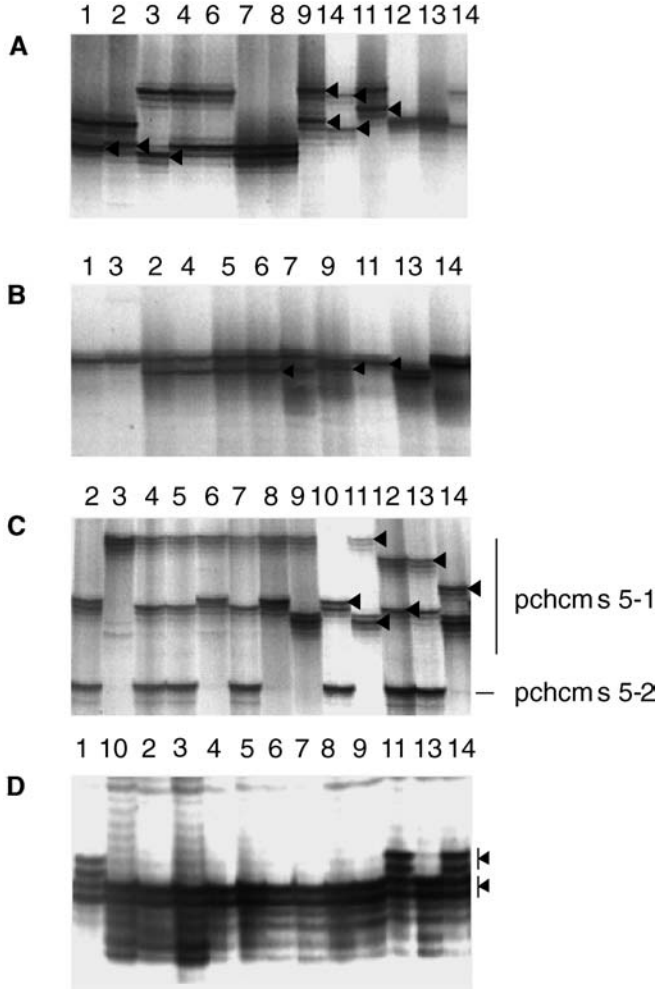

Fig. 1A–D SSR markers pchgms 20 F1, R1 (in panel **A**); pchgms 11 (in panel **B**); pchcms 5 (in panel **C**) and UPD 96-001 (in panel **D**) in hybrid apricot cultivars of known genealogy. *Lanes 1–14* are apricot cultivars: *1* Krimskii Amur; *2* Vynoslyvyi; *3* Shalakh; *4* Lunnik; *5* Shedevr; *6* Olimp; *7* Parnas; *8* Naslazhdenije; *9* Dionis; *10* Krasnoshchekii; *11* Medunets Krimskii; *12* Samyi Rannii; *13* Priusadebnyi; *14* Oranzhevo-krasnyi. The different alleles are indicated by *arrows*

pchcms 5-2 (Fig. 1C). For the polymorphic loci, presence of the nonamplifying (null) alleles was scored in the homozygous state, but they could not be detected in the heterozygous state.

Genetic diversity of SSR markers and genetic identities of cultivars

Fourteen putative SSR loci, amplified by 12 primer pairs, were scored in 74 native apricot germplasm accessions. The number of alleles observed at each locus ranged from two (pchgms 17 and UPD 96-001) to 16 (pchcms 5-1) with an average of 7.64 (Table 3). Altogether, 107 alleles were identified in the set of accessions. Allelic differences were found among apricots that originated from different ecogeographical groups. Allelic variants unique to cultivars from China were revealed at loci pchgms 5-1, pchgms 12, pchgms14, pchgms 20 F1, R1 and pchgms 26 F2, R2-2. No unique alleles were observed in other ecogeographical groups.

Observed heterozygosity (H_o) for individual loci ranged from 0.216 for pchgms 17 to 0.838 for pchgms 3, with an average of 0.517. The discriminating power of loci increases proportionally with increased H_o, and values of this index were high (> 0.7) for five loci (Table 3). On average, and for most individual loci, expected heterozygosity was greater than observed heterozygosity.

The 12 primer pairs utilized in this study unambiguously discriminated all but 11 cultivars. The latter formed three groups, each containing cultivars with indistinguishable genetic profiles. The first group contained two European cultivars, the second group contained six European cultivars and one Irano-Caucasian cultivar, and the third group contained the Ukrainian hybrid cultivar Lunnik and its γ-irradiated mutant Shedevr (Table 4). Genotypes at six of the 14 loci discriminated between the first two groups of widespread related cultivars.

Table 4 Genotypes of apricot cultivars undistinguishable at 14 SSR loci

Locus	Cultivar		
	Ananasnyi Tsurupinskii (Ukraine) Alberge de Tur (France)	Bergeron (France) Luizet Krupnoplodnyi (France) Krasnoshchekii (Ukraine) Yubilejnyi (Ukraine) Nakhichevanskii (Azerbajdzhan) Velkopavlovička (Czech Republic) Vengerskii Krupnyi (Hungary)	Lunnik (Ukraine) Shedevr (Ukraine)
pchgms 3	GJ	GJ	JJ
pchcms 5-1	KL	KL	KV
pchgms 10-1	CC	AC	AC
pchgms 10-2	BB	BB	BB
pchgms 11 F1, R1	CC	AC	AC
pchgms 12	LL	HL	HL
pchgms 14	AA	AB	AB
pchgms 17	BB	BB	BB
pchgms 20 F1, R1	HH	HL	CR
pchgms 20 F1, R2	CC	CC	BF
pchgms 26 F2, R2 –1	AA	AA	BC
pchgms 26 F2, R2 –2	BB	EE	DD
UPD 96-001	AA	AA	AA
UPD 98-406	CJ	CJ	CJ

Genetic relationships among native apricot cultivars based on SSR variation

To elucidate genetic relationships among native apricot cultivars, a dendrogram was produced using neighbor

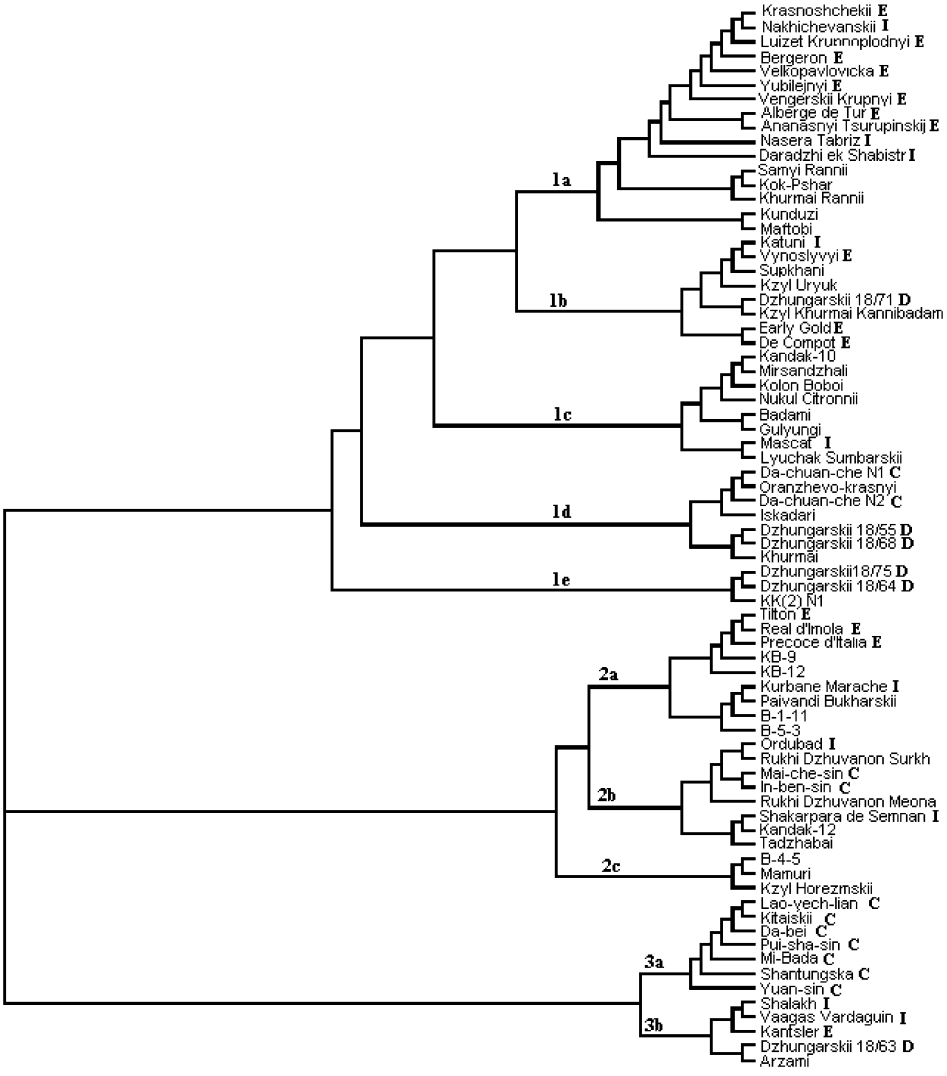

Fig. 2 Neighbor joining dendrogram of genetic distances 74 native apricot varieties based on the data of 14 SSR loci. European (**E**), Irano-Caucasian (**I**), Chinese (**C**) cultivars and Dzungar-Zailij (**D**) forms are marked. Accessions with no label are Central Asian cultivars

joining (NJ) analysis of Nei's pairwise genetic distances over 14 SSR loci. Dendrogram structure was not affected by the order in which samples were analyzed. The cultivars clustered into three main groups, each with subdivision into smaller clusters (Fig. 2). Overall, most European cultivars and Dzhungar-Zailij forms were in the first group, most Central Asian cultivars were in the first and second groups (most Fergana cultivars in the first group), most Chinese cultivars were in the third group, and Irano-Caucasian cultivars were scattered among groups. Within three of the compact clusters, accessions from the same ecogeographical area were grouped together: cluster **1a** contained eight European cultivars (two of the cultivar sets with indistinguishable marker profiles, in Table 4); seven of the eight cultivars in cluster **1c** were Central Asian; and cluster **3a** comprised seven Chinese cultivars.

In general, distribution of the 52 remaining accessions did not reflect their geographical origins or breeding history, with six exceptions noted below. (1) Pedigree relatedness is suspected for the Central Asian (Fergana) cultivars Samyi Rannii, Kok-Pshar and Khurmai Rannii (cluster **1a**). (2) The European cultivars Early Gold, De Compot and Vynoslyvyi, suspected to have Asian ancestors because of sweet kernel, are clustered together (in **1b**) with three cultivars from Central Asia (Fergana). (3) Most of the Dzhungar-Zailij forms are in clusters **1d** and **1e**, together with the widespread Central Asian apricot Khurmai and the half-wild form KK(2)N1. (4) The Chinese cultivars Da-chuan-che N1 and N2 are grouped (in **1d**) with the Central Asian cultivar Iskadari, which is considered to be synonymous with the Chinese cultivar Isko-Dari mentioned by some authors (Mehlenbacher et al. 1990). (5) Cluster **3b**, adjacent to the Chinese cluster **3a**, contains apricots from different geographical areas. However, cultivars Shalakh (leaves) and Arzami (fruits) display the similarity of some anatomical and morphological traits with apricots from China (Kovaljov 1963; Rostova and Sokolova 1992). (6) Pedigree relatedness is known for the Armenian cultivars Shalakh and Vaagas Vardaguin.

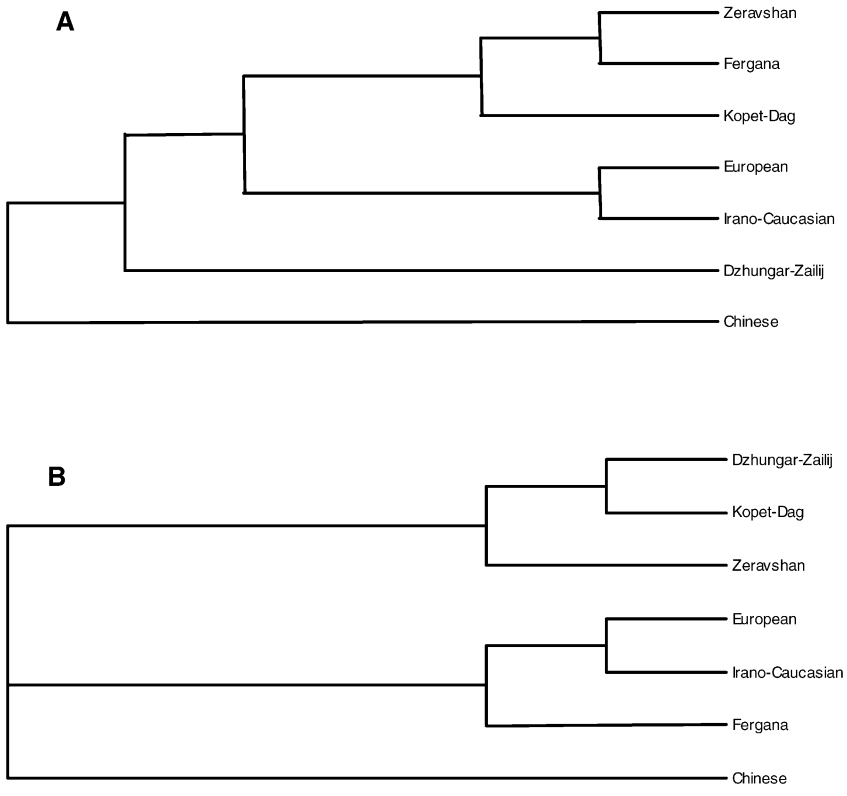

Fig. 3 UPGMA (**A**) and NJ (**B**) dendrograms of relationships among ecogeographical groups and subgroups of cultivated apricots, based on Nei's (1978) genetic distances derived from allele frequencies at 14 polymorphic SSR loci

Table 5 Nei's genetic identity I (the upper triangle) and genetic distance D (the lower triangle) for ecogeographical groups and subgroups of cultivated apricot

Groups/subgroups	European	Irano-Caucasian	Chinese	Fergana	Kopet-Dag	Zeravshan	Dzhungar-Zailij
European	****	0.9409	0.7722	0.8646	0.8330	0.8053	0.7523
Irano-Caucasian	0.0609	****	0.8341	0.9341	0.9165	0.8935	0.8168
Chinese	0.2585	0.1814	****	0.8543	0.8329	0.8410	0.7697
Fergana	0.1455	0.0682	0.1575	****	0.8838	0.9217	0.8539
Kopet-Dag	0.1827	0.0872	0.1829	0.1235	****	0.8936	0.8828
Zeravshan	0.2166	0.1126	0.1732	0.0815	0.1125	****	0.8751
Dzhungar-Zailij	0.2846	0.2024	0.2617	0.1580	0.1247	0.1335	****

Genetic relationships among ecogeographical groups and subgroups of apricot

Based on allele frequencies for the 14 SSR loci, Nei's pairwise genetic identity **I** and genetic distance **D** were calculated for the ecogeographical groups and subgroups (Table 5). Genetic identities were lowest (0.75 to 0.77) among the Chinese, Dzhungar-Zailij and European groups. Highest genetic identities were between the Irano-Caucasian and European groups (0.94) and the Irano-Caucasian group and Fergana subgroup (0.93). Among Central Asian apricots, the highest identity was between the Fergana and Zeravshan subgroups (0.92). Dzhungar-Zailij forms had the strongest affinity with the Kopet-Dag subgroup (0.88).

UPGMA and NJ dendrograms were constructed based on **D**. Structure of the UPGMA dendrogram was not affected by the order in which samples were analyzed (Fig. 3A). In NJ analyses, when sample order was randomized, the dendrogram in Fig. 3B was produced in half of the analyses, and a different dendrogram (not shown) was produced in the others. In all UPGMA and NJ dendrograms, the European and Irano-Caucasian groups clustered together and the Zeravshan and Kopet-Dag subgroups of Central Asia clustered together. In UPGMA analysis (Fig. 3A), the Fergana subgroup was clustered with Zeravshan and Kopet-Dag, and the Chinese group was separate from others, more distantly than the wild Dzhungar-Zailij forms. In half of the NJ analyses (Fig. 3B), the Chinese group was likewise separate from all others, but Fergana was clustered with the European and Irano-Caucasian, and Dzhungar-Zailij was clustered with Zeravshan and Kopet-Dag. In the other NJ analyses (data not shown), Fergana was separate from all others, and Dzhungar-Zailij and Chinese apricots were clustered with Zeravshan and Kopet-Dag.

Discussion

To a certain extent, transportability of SSR markers between plant species reflects their phylogenetic relationships. Cross-species amplification of SSRs is reported to be limited between distantly related crop species in Gramineae (Röder et al. 1995) and among genera within the Brassicaceae (Plieske and Struss 2001). On the other hand, cross amplification confirmed by sequence analysis has been documented between woody perennial species in the genus *Vitis* of Vitaceae (Di Gaspero et al. 2000) and between species of *Malus* and *Pyrus*, in the subfamily Pomoideae of Rosaceae (≥90% sequence identity) (Yamamoto et al. 2001). Our results gave substantial evidence for SSR transportability across species in the Rosaceae. Sixteen of 30 SSR primer pairs, developed from SSR-enriched peach genomic libraries and BAC library clones, generated PCR products in apricot similar in size to the products in peach. Our results correspond with those of Cipriani et al. (1999), who reported that 59% of peach SSR primer pairs amplified products of the expected size in other *Prunus* species, and reflect a high level of homology between SSR flanking regions in the peach and apricot genomes (Wang et al. 2002). Homology between apricot and other *Prunus* species has also been reported when RFLP probes from genomic and cDNA libraries of various *Prunus* species (almond, peach, *Prunus ferganensis,* cherry, plum) were hybridized with heterologous DNA (De Vicente et al. 1998; Joobeur et al.1998; Wang et al. 1998). Thus we expect that SSRs from a model Rosaceae species (such as peach) will be highly effective as anchor loci in comparative mapping between *Prunus* crops.

In our set of apricot cultivars, peach-derived SSR markers detected considerable polymorphism. The mean number of alleles per locus (7.64) is comparable with values reported for other crops such as wheat, with 6.2 (Plaschke et al. 1995); barley, 8.6 (Struss and Plieske 1998); and rapeseed, 4.0 alleles per locus (Plieske and Struss 2001). Levels of polymorphism in our study were considerably higher than 2–4 alleles per locus, reported by Cipriani et al. (1999) and Sosinski et al. (2000) for the predominantly self-pollinating peach. Higher values have been reported for outcrossing, fruit-bearing perennials such as apple, with 8.2 and 12.1 (Gianfranceschi et al. 1998; Hokanson et al. 1998 respectively); sour cherry, 10.7 (Cantini et al. 2001); and grape, 27.4 alleles per locus (Lamboy and Alpha 1998). In our study, observed heterozygosity averaged over the 14 loci was 0.517, higher than the mean value reported for SSRs in peach (0.32, Cipriani et al. 1999) and lower than values detected in apple (0.693, Hokanson et al. 1998; 0.78, Gianfranceschi et al. 1998) and cherry (0.946, Cantini et al. 2001).

High allele number and heterozygosity reflect the ability of SSR markers to provide unique genetic profiles for individual plant genotypes. However, this discriminating ability is much greater with hybrid cultivars than with mutant or clonal cultivars, which cannot be distinguished unless mutations are located within SSR loci or flanking regions. For example, mutant pear cultivars (Yamamoto et al. 2001) and apple sport mutants (Hokanson et al. 1998) were indistinguishable by SSR analysis. On the other hand, SSR markers were successfully employed to decipher homonyms and synonyms in grape germplasm collections (Lopes et al. 1999; Fossati et al. 2001). In our study, we distinguished all cultivars except two pairs and one group of seven (Table 4). AFLP analysis with nine primer pairs failed to further differentiate the European apricots inside of groups (unpublished data), and we suggest that they are synonymous cultivars, variants of a common genotype.

Neighbor joining (NJ) analysis of the cultivars (Fig. 2) produced groups that were not directly based on cultivars' geographic origins, reflecting the complex history of apricot domestication. Cultivars from each eco-geographical area were presented in each of three major dendrogram groups. However, the most common European genotypes were in a group with most Irano-Caucasian, Fergana and Zeravshan cultivars and wild Dzhungar-Zailij forms, and were most distant from a compact group of Chinese cultivars.

According to Vavilov, China and central Asia are two primary centers of apricot domestication. In Central Asia, native cultivars are most likely derived from wild *P. armeniaca*, and co-cultivation of wild and domesticated forms was practisized in the mountainous area from the Kazakhstan-Chinese border (Dzhungar-Zailij) south to Kashmir and west into Afghanistan (Vavilov 1951). Within this area, apricots of the Fergana Valley are richest in diversity and the most representative (Kostina 1931). In our study, the clustering of most Fergana cultivars and wild Dzhungar-Zailij forms with some Chinese cultivars in the first dendrogram group supports this hypothesis.

In ancient China, apricot domestication took place in two separate regions (Mehlenbacher et al. 1990). In the mountains of northern and northeastern China, distribution of wild *P. armeniaca* overlaps with that of *P. sibirica* and *P. mandshurica*. Morphological and physiological traits typical of these two cold-resistant species often are present in cultivars from this area, such as those in cluster **2b**. Alleles characteristic to *P. sibirica* were revealed at isozyme locus *Est-2* and SSR locus pchcms 5-1 in In-ben-sin (unpublished data), and results of isozymes and RAPD analyses support separation of these cultivars in **2b** from the main group of Chinese apricots (Zhebentyayeva and Sivolap 2000). Cultivars from the eastern/central Chinese region: are adapted to a warmer, humid climate; are grown in the same area as *P. mume*, wild forms of which occur in the central Chinese mountains; and have been classified according to morphological traits as *P. armeniaca* var. *ansu* Maxim. (Mehlenbacher et al. 1990) or *Prunus ansu* (Maxim.) Kom. (Kostina 1964; Layne et al. 1996). We believe that the Chinese cultivars in cluster **3a** originated from this region.

A secondary Near-Eastern centre of domestication is the mountainous region from northeastern Iran to the Caucasus Mountain and Central Turkey (Vavilov 1951).

Kostina (1946) suggested that apricots disseminated from central Asia westward through Iran and the Transcaucasian area. Interaction of Central Asian and Chinese apricot germplasm may have taken place in this region, which includes the Kopet-Dag mountains bordering Iran and Turkmenistan (Rostova and Sokolova 1992). The Khorezm oasis, on the Great Silk Road from China to Europe, was another point of germplasm enrichment from primary centers of domestication (Kovaljov 1963). These secondary centers may be represented by the second major group in our dendrogram, which includes most Kopet-Dag and Khorezm cultivars as well as Irano-Caucasian and Chinese.

European apricot cultivars most likely originated from relatively few forms brought from the Near-Eastern region. In our study, this is supported by the grouping of several European cultivars with those from Irano-Caucasia and Kopet-Dag (Fig. 2, clusters **1a, 2a** and **3b**) and the clustering of ecogeographical groups (Fig. 3). Six common European cultivars had the same SSR allelic profile as one Irano-Caucasian cultivar (**1a**) and the same isozymes profile as a widespread Central Asian cultivar, Safidak (unpublished data). Layne et al. (1996) suggested that the Romans knew only early ripening forms of apricot, possibly similar to the early ripening Fergana cultivars in cluster **1a** (Fig. 2). We hypothesize that most common European genotypes originated in Central Asia, reached Europe through the Caucasian region (e.g., as Nakhichevanskii; see Table 4) and spread over a large area. Further evidence of synonymous Asian and European cultivars is needed to support this.

Despite the polymorphism detected among cultivars, overall variation between versus within the ecogeographical groups was low. Distant positioning of the Chinese group on UPGMA and NJ dendrograms (Figs. 2 and 3) supports separating eastern Chinese apricots from other germplasm, but high genetic identities between the groups (> 0.75) justifies considering this group as subspecies *P. armeniaca* var. *ansu*, not as a separate species. For comparison, interspecies similarities in *Avena* (SSR study) and in *Olea* (AFLP study) were <0.46 and ≤0.56 respectively (Angililo et al.1999; Li et al. 2000).

Traditional classification of apricot germplasm into four ecogeographical groups and regional Central Asian subgroups, based on physiological and morphological differences (Kostina 1936, 1964), was supported by UPGMA cluster analysis of genetic distances between the groups (Fig. 3A). However, NJ cluster analysis of the distances between groups and among individual cultivars (Fig. 2 and Fig. 3B) did not support integration of Fergana germplasm with the other Central Asian subgroups, instead clustering them with the wild Dzhungar-Zailij forms. These discrepancies may result from examining only a limited number of cultivars representing the Central Asian subgroups. However, we believe that the NJ dendrogram reflects the true patterns of relatedness between apricot germplasm groups. Independent positioning of Fergana varieties within Central Asian germplasm was revealed by multi-factorial analysis of anatomical and morphological leaf characters (Rostova and Sokolova 1992); furthermore, Avdeev (1992) proposed that the Kopet-Dag subgroup represents relic center of primitive apricots propagated by seeds because of similarity of some morphological and physiological traits with that of wild *P.armeniaca*. In Central Asia, domestication may have occurred through the natural association of wild and half-wild forms and their interaction with cultivated germplasm within the region, originating from the Fergana and Chinese centers of domestication. The strong affinity between Fergana, European and Irano-Caucasian cultivars supports the hypothesis that Fergana germplasm had a dominant influence on dispersion of apricots throughout the world. Further analysis of additional germplasm representing each region, particularly Fergana and Chinese cultivars, may help us examine this hypothesis and identify traits and genes that contributed to the fitness and dispersion of apricot cultivars from these regions.

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References

Avdeev VI (1992) On the centres of provenance of cultivated apricot. Bull Appl Bot Genet Plant Breed 146:33–35

Angililo A, Mencuccini M, Baldoni L (1999) Olive genetic diversity assessed using amplified fragment length polymorphism. Theor Appl Genet 98:411–421

Badenes ML, Asíns MJ, Carbonell EA, Glácer G (1996) Genetic diversity in apricot, *Prunus armeniaca* L. aimed at improvement of resistance to plum pox virus. Plant Breed 115:133–139

Battistini S, Sansavini S (1991) Electrophoretic analysis of isozyme variability in apricot germplasm. J Genet Breed 45:117–122

Byrne DH, Littleton TG (1989) Characterization of isozyme variability in apricots. J Am Soc Hort Sci 114:674–678

Cantini C, Iezzoni AF, Lamboy WF, Boritzki M, Struss D (2001) DNA fingerprinting of tetraploid cherry germplasm using simple sequence repeats. J Am Soc Hort Sci 126:205–209

Cipriani G, Lot G, Huang W-G, Marazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: isolation, characterization and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

De Vicente MC, Truco MJ, Egea J, Burgos L, Arus P (1998) RFLP variability in apricot (*Prunus armeniaca* L.). Plant Breed 117:153–158

Di Gaspero G, Peterlunger E, Testolin R, Edwards KJ, Cipriani G (2000) Conservation of microsatellite loci within the genus *Vitis*. Theor Appl Genet 101:301–308

Felsenstein J (1989) PHYLIP – Phylogeny Inference Package. Cladistics 5:164–166

Gianfranceschi L, Seglias N, Tarchini R, Komjanc M, Gessler C (1998) Simple sequence repeats for the genetic analysis of apple. Theor Appl Genet 96:1067–1076

Fossati T, Labra M, Castiglione S, Failla O, Scienza A, Sala F (2001) The use of AFLP and SSR molecular markers to decipher homonyms and synonyms in grapevine cultivars: the case of the varietal group known as "Schiave". Theor Appl Genet 102:200–205

Hokanson SC, Szewc-McFadden AK, Lamboy WF, McFerson JR (1998) Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus* × *domestica* Borkh. core subset collection. Theor Appl Genet 97:671–683

Hormaza JI (2002) Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. Theor Appl Genet 104:321–328

Joobeur T, Viruel MA, De Vicente MC, Jáuregui B, Ballester J, Detorri MT, Verde I, Truco MJ, Messeguer R, Batlle I (1998) Construction of a saturated linkage map for *Prunus* using an almond × peach F2 progeny. Theor Appl Genet 97:1034–1041

Karp A, Seberg O, Buatti M (1996) Molecular techniques in the assessment of botanical diversity. Ann Bot 78:143–149

Kostina KF (1931) The cultivation of apricots in Fergana Valley (in Russian). Bull Appl Bot Genet Plant Breed 27:3–158

Kostina KF (1936) The apricot (in Russian). Bull Appl Bot Gen Plant Breed Suppl 83. Institute of Plant Industry, Leningrad

Kostina KF (1946) The origin and evolution of cultivated apricot (in Russian). Trud Nikit Bot Gard 24:25–31

Kostina KF (1964) Application of phytogeographical method to apricot classification (in Russian). Trud Nikit Bot Sad 37. Kolos, Moscow

Kovaljov NV (1963) The Apricot (in Russian). Selchozizdat, Moscow

Lamboy WF, Alpha CG (1998) The utility of simple sequence repeats (SSRs) for DNA fingerprinting germplasm accessions of grape (*Vitis* L.) species. J Am Hort Sci USA 123:182–188

Layne REC, Bailey CH, Hough LF (1996) Apricots. In: Janick J, Moore JN (eds) Fruit breeding, volume 1: tree and tropical fruits. John Wiley and Sons, New York, pp 79–111

Li CD, Rossnagel BG, Scoles GJ (2000) The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor Appl Genet 101:1259–1268

Lopes MS, Sefc KM, Eiras Dias E, Steinkellner H, Laimer da Câmara Machado M, da Câmara Machado A (1999) The use of microsatellites for germplasm management in a Portuguese grapevine collection. Theor Appl Genet 99:733–73

Martinez-Gomez P, Arulsekar S, Potter D, Dejong T, Gradziel TM, Bliss FA (2001) SSR variation within and between peach and almond cultivars. Plant and Animal Genome IX, January 13–17, 2001, San Diego, California P541

Mehlenbacher SA, Cociu V, Hough LF (1990) Apricots (*Prunus*). In: Moore JN, Ballington JR (eds) Genetic resources of temperate fruit and nut crops. International Society for Horticultural Science, Wageningen, The Netherlands, pp 63–108

Minch E (1997) MICROSAT, version 1.5d. Stanford University Medical Center, Stanford, California

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12: 357–358

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingley S, Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed 2:225–238

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Plieske J, Struss D (2001) Microsatellite markers for genome analysis in *Brassica*. Development in *Brassica napus* and abundance in *Brassicaceae* species. Theor Appl Genet 102:680–694

Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001) Two genetic linkage maps of tetraploid roses. Theor Appl Genet 103:575–583

Rehder A (1967) Manual of cultivated trees and shrubs, 2nd edn. Macmillan, New York

Röder MS, Plaschke J, König SU, Börner A, Sorrells E, Tanksley SD, Ganal M (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246:327–333

Rostova NS, Sokolova EA (1992) Variability of anatomical and morphological leaf characters in apricot (*Armeniaca* Scop.) species and varieties (in Russian). Bull Appl Bot Genet Plant Breed 146:74–86

Sivolap YuM (ed) (1998) Application of PCR analysis to genetic research and breeding (in Russian). Agrarna nauka, Kiev

Sosinski B, Gannavarapu M, Hager LD, Beck LE, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theor Appl Genet 101:421–428

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97:308–315

Takeda T, Shimada T, Nomura K, Ozaki T, Haji T, Yamaguchi M, Yoshida M (1998) Classification of apricot varieties by RAPD analysis. J Jpn Soc Hort Sci 67:21–27

Vavilov NI (1951) Phytogeographical basis of plant breeding. In: Chester KS (trans) The origin, variation, immunity and breeding of cultivated plants. Chron Bot 13:13–54

Wang D, Kartle R, Brettin TS, Iezzoni AF(1998) Genetic linkage map in sour cherry using RFLP markers. Theor Appl Genet 97:1217–1224

Wang Y, Georgi LL, Zhebentyayeva TN, Reighard GL, Scorza R, Abbott AG (2002) High-throughput targeted SSR marker development in peach [*Prunus persica* (L.) Batsch]. Genome 45:319–328

Yamamoto T, Kimura T, Sawamura Y, Kotobuki K, Ban Y, Hayashi T, Matsuta N (2001) SSRs isolated from apple can identify polymorphism and genetic diversity in pear. Theor Appl Genet 102:865–870

Yang R-C, Yeh FC (1993) Multilocus structure in *Pinus contorta* Dougl. Theor Appl Genet 87:568–576

Zhebentyayeva TN, Sivolap YuM (2000) Genetic diversity of apricot determined by isozyme and RAPD analyses. Acta Hort 538:525–529