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Genetic Diversity and Identity of Chinese Loquat Cultivars/Accessions (*Eriobotrya japonica*) Using Apple SSR Markers

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Abstract Loquat (*Eriobotrya japonica*) is an underutilized fruit crop that originated in China and for which only a small number of molecular markers are available. This number can be increased by identifying apple SSRs that are transferable to loquat cultivars/accessions to provide new insight into the level of genetic diversity within loquat and synteny with apple. We evaluated 71 apple SSR markers distributed across 17 linkage groups, and identified 39 SSRs transferable to loquat. Testing 54 loquat accessions, from Japan, Spain, four provinces in China, and two wild species gave a total of 155 different alleles with a mean value of 3.38 per locus. The mean effective number of alleles was 2.21, and the mean observed heterozygosity was 0.47. These values indicate a high degree of genetic diversity in the set of Chinese loquat accessions analyzed. Unweighted pair-group method analysis based on simple matching coefficent clustered the accessions into two groups, cultivated and wild loquat. The cultivated loquat can be subdivided into three subgroups which generally reflect their geographic origin in China. The Spanish cultivars clustered with those of the Jiangsu and Zhejiang provinces. A core set of five SSR markers could distinguish most accessions.

Keywords *Eriobotrya japonica* · Genetic diversity · Simple sequence repeat · Genetic relationship

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Introduction

Loquat [*Eriobotrya japonica* (Thunb) Lindl.], a subtropical evergreen fruit tree of the Rosaceae subfamily Maloideae, originated in southwest China and has been cultivated for over 2,000 years (Qiu and Zhang 1996). It has been introduced to more than 30 countries including Japan, Mediterranean countries in Europe, India, Australia, New Zealand, Madagascar, and South Africa, while commercial cultivation is limited to a few countries. In China, loquat blooms in the fall and early winter, and the fruit ripens between May and June. Loquat leaves and fruits are traditionally used for treating coughs and as expectorant, and the flower is an excellent source of honey. In 2005, around 131,000 ha were planted worldwide (120,000 ha in China), with a production of more than 549,220 t (400,000 t in China). In China, the Sichuan, Fujian,

Jiangsu, and Zhejiang provinces are the main production areas (Lin 2007). The loquat cultivars from these areas are the most well-known and commonly planted in China, mainly 'Dawuxing', 'Longquan No.1', 'Zaozhong No.6', 'Jiefangzhong', 'Dahongpao', 'Luoyangqing', 'Ruantiaobaisha', 'Baiyu', 'Qingzhong' and 'Guanyu'.

The pedigrees of the majority of loquat cultivars are unknown as historically. The current system used for cultivar classification gives little information on genetic identity and variability as it is mainly based on morphological traits linked to ecotype, flesh color, fruit shape, usage, and ripening time (Martínez-Calvo et al. 2008). Genetic diversity and the relationships among different cultivars of loquat are of great importance for the conservation of genetic resources, breeding initiatives, and national and international exchange of materials.

Using molecular markers has significantly contributed to our understanding of genetic diversity and relatedness in various crops. Microsatellites or simple sequence repeats (SSRs) are markers generally used to detect polymorphism (Weber and May 1989). The applicability of SSR markers for apple, loquat, and peach has been reported (Soriano et al. 2005; Qiao 2008; Watanabe et al. 2008; Gisbert et al. 2009a/b). Soriano et al. (2005) used 30 SSRs from apple to assay the genetic relationship in loquat, 13 of which amplified polymorphic products and distinguished 34 of the 40 loquat accessions originating from different European countries. SSR markers have also been used to identify the polyploidy level of loquat (Watanabe et al. 2008). Recently, Gisbert et al. (2009a) constructed the first loquat linkage maps with 83 SSRs and 94 AFLPs, which will also be very useful in genetic analysis and comparative genome research with other Rosaceae fruits. However, an in-depth analysis of the genetic diversity of loquat species, using large numbers of SSR markers and accessions from different geographical areas of the world, is still lacking.

In this paper, we tested the transferability of 71 apple SSRs (Silfverberg-Dilworth et al. 2006; Gisbert et al. 2009a) to loquat to study the genetic diversity of, and fingerprint, 51 loquat accessions of local cultivars from the Jiangsu and Zhejiang and other Chinese provinces, and three cultivars from Spain and Japan.

Materials and Methods

Plant Materials

Fifty two loquat cultivars/accessions were examined (Table 1), of which 27 (including the Japanese cultivar 'Moriowase') were from the Taihu Extension Center for Evergreen Fruit (Jiangsu province), 14 from the Yuhang District Loquat Institute (Zhejiang province), six from Fujian Putian College, and five from an orchard at Southwest University. In addition, two DNA sample of Spanish cultivars 'Marc' and 'Peluches' were provided by Professor Shunquan Lin at the South China Agricultural University. The two wild species, 'Liye' (*Eriobotrya prinoides* var. prinoides) and 'Daduhe' (*E. prinoides* var. daduheensis, a natural hybrid of *E. prinoides* var. prinoides and *E. japonica*) accessions of the native wild species *E. prinoides* were included as an outgroup reference. The geographical distribution of Chinese accessions is shown in Fig. 1, and some of the cultivars with known genetically relationship shown in Fig. 2

DNA Extraction and Examination

DNA extraction was based on the method of Roche et al. (1997), modified by adding 0.1 g PVP powder to grind the leaf material. After extraction, 2-5 μl DNA solution was loaded on 1.0% agarose gel to check the quality.

SSR Primers

We selected a total of 71 SSR markers, including six from Soriano et al. (2005), covering the 17 linkage groups in apple (Silfverberg-Dilworth et al. 2006; Patocchi et al. 2008), with at least two well-separated loci from each linkage group. The primer sequences and polymerase chain reaction (PCR) conditions were obtained from the apple SSR database (http://www.hidras.unimi.it) (Gianfranceschi and Soglio 2004). Primers were synthesized by Shanghai Invitrogen™ Life Technologies.

PCRs and Electrophoresis

PCR amplification was in a total volume of 20 μl, containing: 10 ng genomic DNA, 2 μl of 10× PCR buffer (with $MgCl_2$), 0.1 μl of 10 mM dNTP mixture, 0.5 μl each of forward and reverse primer (10 pmol/μl), and 0.5 U *Taq* polymerase (Takara Biotechnology Company, Dalian). The reactions were performed using an Eppendorf Mastercycler (Gradient, No. 5331-41264, Germany) with the following conditions: 94°C for 2 min and 30 s, then 35 cycles of 94°C for 30 s, 50-62°C for 30 s, and 72°C for 30 s, and a final step at 72°C for 10 min. The annealing temperature for each primer pair was optimized using a PCR gradient (T=57°C, R=3°Cs, G=8°C). Twenty microliter PCR products were mixed with 5 μl formamide loading buffer (98% formamide, 10 mM EDTA, 0.25% bromophenol blue, 0.25% xylene cyanol, pH8.0), heat-denatured at 95°C for 5 min, and 5 μl of each mixture and a molecular size marker of *pBR*322 DNA-*Msp*I Digest (New England BioLabs) loaded onto a 6% denaturing polyacrylamide gel (7 M urea) in 1× TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH8.0). Gels were run at 60 W, 45°C for 1 to 1.5 h in a sequencing gel

Table 1 Loquat accessions used in this study; parentage is unknown, unless stated otherwise

Cultivar/accession	Description	Acronym[a]
Bahong	Orange-red pericarp, red fleshed	JS-BH
Baili	Yellow or orange-yellow pericarp, white fleshed	FJ-BL
Baisha No.2	White fleshed	JS-BS2
Baiyu	Orange-yellow pericarp, white fleshed, seedling progeny of 'Zaohuang'	JS-BY
Baozhu	Yellow or orange-yellow pericarp, red fleshed	ZJ-BZ
Bingtangzhong	Yellow-white pericarp, white fleshed	JS-BTZ
Biqizhong	Yellow-orange pericarp, white fleshed, fruit with appearance of water chestnut, seedling progeny	JS-BQZ
Changhong	Orange-red pericarp, red fleshed	FJ-CH
Changlv No.2	Yellow-white pericarp, white fleshed	JS-CL2
Changlv No.3	White fleshed	JS-CL3
Changlv No.4	Orange-yellow pericarp, white fleshed, large fruit derived from cv. 'Baisha'	JS-CL4
Changlv No.5	White fleshed, progeny of 'Baiyu'×'Tianzhong'	JS-CL5
Changlv No.6	White fleshed, seedling progeny of 'Guanyu'	JS-CL6
Chihong	Orange-red pericarp, red-fleshed, seedling progeny of 'Dahongpao'	ZJ-CH
Chuannao	Orange-yellow pericarp, white fleshed	JS-CN
Daduhe	*E. prinoides* var. *Daduheensis*, red fleshed, small fruit, wild species, natural hybrid of *E. prinodides* var. *prinoides* and *E. japonica* (Thunb) Lindl[b]	SC-DDH
Dahongpao	Orange-red pericarp, red fleshed, deep orange-red pericarp and pulp	ZJ-DHP
Dahongsha	Red fleshed	JS-DHS
Dameiguihongpao	Orange-red pericarp, red fleshed, seedling of 'Dahongpao'	ZJ-DMGHP
Dawuxing	Orange-yellow pericarp, red fleshed, with star like navel	SC-DWX
Dayeyangdun	Orange-yellow pericarp, red fleshed	ZJ-DYYD
Dazhong	White fleshed	JS-DZ
Dongshanjidanbai	White fleshed	JS-DSJDB
Erzao	Orange-yellow pericarp, red fleshed	ZJ-EZ
Gaoliangjiang	Orange-yellow pericarp, white fleshed	JS-GLJ
Guanyu	White fleshed, seedling progeny of Baisha loquat	JS-GY
Hongmao	Red fleshed	JS-HM
Jiajiao	Yellow pericarp, red fleshed	ZJ-JJ
Jidanbai	Yellow pericarp, white fleshed	JS-JDB
Jidanhong	Orange-yellow pericarp, red fleshed, seedling progeny	JS-JDH
Jiefangzhong	Orange-red pericarp, red fleshed, seedling progeny of 'Dazhong'	FJ-JFZ
Jinfeng	Orange-yellow pericarp, red fleshed	SC-JF
Liufenzhong	Orange-yellow pericarp, red fleshed	ZJ-LFZ
Liye	*E. prinoides* var. *Prinoides*, red fleshed, small fruit, wild species	SC-LY
Longquan No.1	Orange-yellow pericarp, red fleshed	SC-LQ1
Luoyangqing	Orange-yellow pericarp, red fleshed, sepal around areas still green when ripe	ZJ-LYQ
Marc	Orange-yellow pericarp, red fleshed	SP-MARC
Meiyu	White fleshed, seedling of Baisha loquat	JS-MY
Moriowase	Orange-red pericarp, red fleshed, branch mutation of 'Mogi'	JP-MW
Peluches	Orange-yellow pericarp, red fleshed, probably Algerie mutation	SP-PC
Qingzhong	Orange-yellow pericarp, white fleshed, seedling progeny of Baisha loquat, early mature fruit with green pedicle	JS-QZ
Ruantiaobaisha	Yellow pericarp, white fleshed	ZJ-RTBS
Taicheng No.4	Orange-red pericarp, red fleshed, seedling progeny	FJ-TC4
Tangkebairou	Orange-yellow pericarp, white fleshed, large fruit, mutation of Baisha loquat	ZJ-TKBR
Tianzhong	Yellow-white pericarp, white fleshed	JS-TZ
Tongpi	Yellow pericarp, white fleshed	JS-TP
Touzao	Orange-yellow pericarp, red fleshed	ZJ

Table 1 (continued)

Cultivar/accession	Description	Acronym[a]
Wuerbaisha	Yellow pericarp, white fleshed, high yield, similar to 'Ruantiaobaisha'	ZJ-WRBS
Xiangzhong	Orange-yellow pericarp, red fleshed, fruit with deep aroma, 'Xiangtian'×'Jiefangzhong'	FJ-XZ
Xiaobaisha	Orange-yellow pericarp, white fleshed	JS-XBS
Yingtiaobaisha	Orange-yellow pericarp, white fleshed, similar to 'Ruantiaobaisha'	ZJ-YTBS
Zaohuang	Orange-yellow pericarp, white fleshed	JS-ZH
Zaozhong No. 6	Orange-yellow pericarp, red fleshed, 'Jiefangzhong'×'Moriowase'	FJ-ZZ6
Zhaozhong	Yellow pericarp, white fleshed, seedling progeny of 'Baisha'	JS-ZZ

[a] The first two capital letters indicate the original provinces in China; *ZJ* Zhejiang, *JS* Jiangsu, *FJ* Fujian, *SC* Sichuan, or *SP* for Spain and *JP* for Japan. This is followed by the acronym for the accession

[b] Proposed by Yang et al. (2007) and Tang (1997)

electrophoresis apparatus (DYCZ-20C, Beijing Liuyi Instrument Factory, China) and silver stained (Brant 1991).

Allele Data and Statistic Analysis

Allele sizes were estimated by comparison to *pBR*322 DNA-*Msp*I Digest, and named in alphabetical order (A, the largest). Where only one band was present, this allele was assumed to be homozygous. Cultivars with known pedigree (Fig. 2) were used to evaluate the scores and the validity of the parentages. All accessions, except the wild species, were analyzed as a single population for number of alleles per locus (Na), effective number of alleles (Ne), Shannon index (*I*), Nei's expected heterozygosity (He), observed heterozygosity (Ho), and Wright's fixation index (*F*). The chi-square test for Hardy–Weinberg equilibrium (P_{hw}) of primers was using the POPGENE program (version 1.32), with 0.01 significance and 10,000 times simulation. A dendrogram with all 54 accessions, based on the matrix of the genetic identities, was constructed using unweighted pair-group method using arithmetic average (UPGMA) with the NTSYSpc 2.10e software (Rohlf 2000). The data were transformed to a matrix of similarity coefficients using simple matching (SM) coefficient. The Eigen procedure of NTSYSpc 2.10e software was used for principal component analysis.

Results

Screening Suitable SSR Markers and Their Variation

Of the 71 apple SSR primer pairs tested, more than half were found to be transferable to loquat: 39 gave polymorphic alleles in loquat (Table 2), 16 get a single or two alleles in all accessions (Table 3), and 16 primers produced

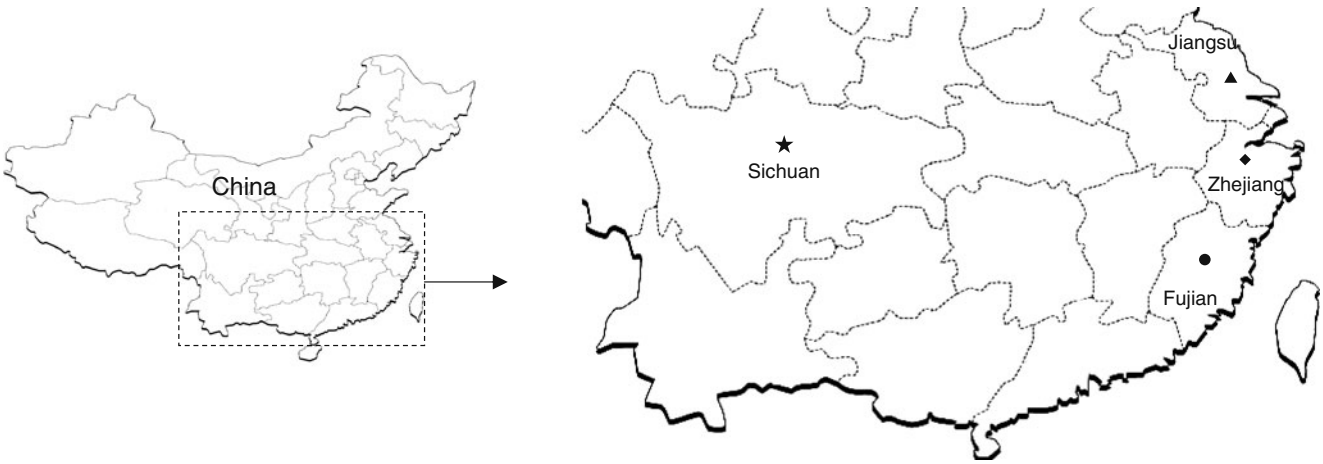

Fig. 1 Map of China indicating the region where the loquat accessions originated. *Filled diamond* Zhejiang, *filled circle* Fujian, *filled triangle* Jiangsu, and *filled star* Sichuan province, respectively

Fig. 2 Illustration of known pedigrees of some accessions. Information is from Table 1, X indicates crossing or seeding progeny, thundering symbol indicates mutant

smear bands. Four markers (AT000400, CH01d03, CH01h02, and CH03c02) were judged to be multi-locus as they amplified more than three alleles in most accessions. The size range of the alleles in loquat differed from that of apple. The 39 polymorphic SSR markers gave a total of 155 alleles, two to seven alleles per marker with an average of 3.38. The effective number of alleles varied from 1.04 for CH01f02 to 4.00 for CH02d12, with an average of 2.21. The Shannon index, as a measure of gene diversity, ranged from 0.10 for CH01f02 to 1.49 for CH03a09, with an average of 0.84. The Ho ranged from approximately zero for CH01f02 to 0.83 for Hi08a04, with a mean of 0.47. Similar values were calculated for He. The fixation index (F) ranged from −0.70 for Hi08a04 to 1.00 for CH01f02, with an average of 0.07. There was significant deviation from the HW equilibrium ($P<0.05$) for 20 loci (Table 2).

In the dendogram constructed from UPGMA cluster analysis of the similarity matrix, with 155 SSR alleles, the accessions are clustered in two groups, the commonly cultivated loquats and the two wild species (Fig. 3) at a 0.48 threshold of SM coefficient. The cultivated loquat accessions were further subdivided into three subgroups (A-C), at a threshold of 0.723, and generally reflected their geographic origin. Subgroup A included all local accessions from the geographically close Zhejiang and Jiangsu provinces, being temperate zone accessions, plus the 'Longquan No.1' from Sichuan and 'Changhong' from Fujian, and the two Spanish cultivars. Subgroup B included the subtropical Fujian cultivars ('Zaozhong No.6', 'Jiefangzhong', 'Xiangzhong', 'Taicheng No.4' and 'Baili'), but also the Sichuan cultivar 'Jinfeng' and the Japanese cultivar 'Moriowase'. The third subgroup included only one cultivar: 'Dawuxing' of Sichuan. The clusters thus generally reflected the geographic origin of their members.

PCA analyses, carried out using the similarity matrices for the 39 SSR markers (Fig. 4), confirmed the UPGMA cluster analysis. The two wild species (group V) were separated from the cultivated accessions, and the cultivated accessions were classified in four groups, mainly according to their geographical distribution. All the Zhejiang accessions, ten Jiangsu accessions, the Japanese cultivar 'Moriowase', and two Spanish cultivars, 'Marc' and 'Peluches', were in group I. The 15 Jiangsu accessions clustered in group II, all the Fujian cultivars in group III, and 'Dawuxing' and 'Longquan No.1', the two most commonly cultivated varieties in Sichuan province, in group IV. The first two principal components explained 9.86% and 8.63% of the total variation, respectively.

Compared to the cultivated varieties, half the markers gave distinct alleles with the wild species 'Liye', while 'Daduhe' had more common alleles, in agreement with its origin as a hybrid between a wild, *E. prinoides* var. prinoide, and cultivated loquat (Table 1).

Genetic Identity of Different Accessions

Thirty nine SSR markers distinguished all accessions except four cultivars: the two Spanish cultivars 'Marc' and 'Peluches' could not be distinguished from each other, and 'Meiyu' could not be distinguished from 'Changlv No.2'. The scores of these markers confirmed pedigrees (Fig. 2), such as that of 'Zaozhong No.6' (a 'Jiefangzhong' × 'Moriowase') and 'Changlv No.5' (a 'Baiyu' × 'Tianzhong'), and 'Xiangzhong' was confirmed as a descendant of 'Jiefangzhong', since for each SSR marker one of the alleles of 'Jiefangzhong' was present in 'Xiangzhong'. 'Moriowase' was also confirmed as a sport of 'Mogi' by comparing published marker scores for Mogi (Watanabe et al. 2008) with our scores for Moriowase (our study did not include Mogi). Identical alleles were found for the three common SSR: 140/140 for CH03a09, 185/185 for CH05a04, and 212/216 for CH02d10a.

For genetic identity comparison, it is important to discriminate the greatest number of accessions with the least number of markers. Based on the number of effective alleles, we selected a set of five SSR markers (CH03a09, CH02c06, CH04g12, CH02d12, and CH05h05) able to distinguish all the accessions except bud sports.

Discussion

We used comparative genomics to transfer apple SSR markers for genetic diversity and identity studies in Chinese loquat. Cultivar grouping based on SSR markers reflected their geographic origin, and a core set of five polymorphic SSR markers allowed efficient and easy identity assessments of the cultivars. The relationship between the cultivated loquat and closely related wild species was also addressed. This research will help to construct better reference linkage mapping populations.

Table 2 List of the genetics parameters of 39 primer pairs in alleles of 52 cultivated loquat accessions (not including wild species)

Locus name	LG in apple	Size range in apple (bp)	Alleles in apple	LG in loquat[f]	Size range in loquat (bp)	Na	Ne	*I*	He	Ho	*F*	P_{hw}
CH-vf1[a]	1	129-174	-[h]	1	146-160	4	2.71	1.12	0.64	0.48	0.24	0.00[g]
AT000400[c]	2	198-232	7	NL	230-233	4	-[h]	-[h]	-[h]	-[h]	-[h]	-[h]
CH02c06[b,d]	2	216-254	3	NL	160-190	2	1.81	0.64	0.45	0.56	−0.25	0.08
CH02f06[b,d]	2	138-157	2	NL	118-121	2	1.99	0.69	0.50	0.50	−0.01	0.98
CH03d10[b]	2	166-182	6	2	154-160	3	2.24	0.88	0.56	0.79	−0.43	0.00[g]
CN493139[c]	2	124-162	10	NL	150-156	4	1.55	0.69	0.36	0.23	0.35	0.00[g]
AU223657[c]	3	219-233	6	NL	240-250	3	1.61	0.64	0.38	0.42	−0.12	0.74
CH01c08[b]	NL	130-228	5	3	118-134	3	1.91	0.77	0.48	0.42	0.11	0.00[g]
Hi15h12[c]	3	222-228	3	3	230-241	4	2.30	1.05	0.57	0.63	−0.12	0.01[g]
CH01b12[b]	4/12/13	125-178	9	10[e]	160-180	2	1.88	0.66	0.47	0.29	0.38	0.00[g]
CH01d03[b]	4/12	136-160	5	NL	141-150	4	-[h]	-[h]	-[h]	-[h]	-[h]	-[h]
Hi23d11[c]	4	177-184	4	4	186-190	2	1.94	0.68	0.49	0.33	0.33	0.012[g]
CH03a09[b]	5	125-143	6	5	136-152	6	4.01	1.49	0.76	0.56	0.26	0.00[g]
Hi08a04[c]	5	246-254	4	NL	325-370	2	1.94	0.68	0.49	0.83	−0.70	0.00[g]
CH03d07[b]	6	186-226	8	NL	290-306	2	1.91	0.67	0.48	0.67	−0.41	0.00[g]
Hi03a03[c]	6	160-228	10	6	183-190	3	2.27	0.92	0.57	0.50	0.11	0.52
U78949[c]	6/14	172-225	12	6	200-210	2	1.40	0.46	0.29	0.23	0.19	0.14
CH04e05[b]	7	174-227	8	7	355-380	3	1.48	0.54	0.33	0.25	0.23	0.27
CH01f09[b,d]	8	112-139	6	NL	128-138	2	1.99	0.69	0.50	0.56	−0.12	0.43
CH01h10[b]	8	94-114	5	8	98-128	2	1.94	0.68	0.49	0.56	−0.15	0.31
AJ320188[c]	9	191-245	7	9	206-209	2	1.12	0.22	0.11	0.12	−0.06	0.69
CH01f03b[b]	9	139-183	7	9	180-193	2	1.58	0.55	0.37	0.33	0.10	0.41
CH01h02[b,d]	9	226-252	3	NL	222-241	4	-[h]	-[h]	-[h]	-[h]	-[h]	-[h]
CH01f07a[b]	10	174-206	8	NL	178-195	3	1.98	0.73	0.51	0.69	−0.40	0.03[g]
CH04g09[b]	10	141-177	11	10	133-163	5	3.88	1.47	0.75	0.77	−0.04	0.00[g]
MS06g03[b]	10	154-190	9	NL	160-166	3	1.41	0.52	0.30	0.04	0.87	0.00[g]
CH02d12[b,d]	11	175-205	5	11	221-242	5	4.00	1.49	0.76	0.75	0.00	0.01[g]
CH01f02[b,d]	12	168-222	3	NL	160-162	2	1.04	0.10	0.04	0.00	1.00	0.00[g]
CH03c02[b]	12	116-136	5	12	120-153	4	-[h]	-[h]	-[h]	-[h]	-[h]	-[h]
CH04g04[b]	12	170-186	5	12	166-192	6	3.82	1.47	0.75	0.50	0.32	0.00[g]
CH05h05[b]	13	168-184	4	NL	165-202	7	3.22	1.40	0.70	0.60	0.14	0.03[g]
Hi04g05[c]	13	190-258	8	13	234-240	3	2.02	0.85	0.51	0.48	0.05	0.10
CH04c07[b]	14	98-135	8	14	105-123	3	2.08	0.77	0.52	0.42	0.18	0.23
CH01d08[b]	15	238-290	6	14[e]	277-300	3	1.79	0.79	0.45	0.46	−0.05	0.63
CH02c09[b]	15	233-257	6	15	258-264	2	1.48	0.50	0.33	0.40	−0.25	0.08
CH02d10a[b]	16	215-229	5	16	206-216	5	3.70	1.41	0.74	0.69	0.05	0.00[g]
CH05a04[b]	16	159-189	8	NL	170-187	5	1.91	0.97	0.48	0.21	0.56	0.00[g]
CH04c10[b]	17	133-180	7	17	107-134	3	2.05	0.88	0.52	0.60	−0.16	0.46
CH04g12[b]	NL	141-186	8	NL	136-180	6	3.24	1.37	0.70	0.52	0.25	0.00[g]
Mean						3.38	2.21	0.84	0.50	0.47	0.07	0.18

NL not located

[a] Vinatzer et al. (2004)

[b] Liebhard et al. (2002)

[c] Silfverberg-Dilworth et al. (2006)

[d] Markers used by Soriano et al. (2005)

[e] Different linkage group between apple and loquat

[f] According to Gisbert et al. (2009a).

[g] Significant deviation from HW equilibrium ($P<0.05$)

[h] Not determined

Table 3 List of the primer pairs that amplified the same bands or no products in all loquat accessions

Locus name	Allele size in apple (bp)	No. of alleles in Apple	Allele size in loquat (bp)	LG in apple
CH03g12[a]	154-200	10	167	01
CH05e03[a]	158-190	10	180/295	02
CH03g07[a]	119-171	5	NP	03
CH04e02[a]	143-163	6	190/255	04
Hi23g02[b]	230-257	6	355	04
AT000420[b]	189-209	5	197/209	04
CH02h11a[a]	104-132	8	NP	04
CH04e03[a]	179-222	11	182	05[d]
CH05b06[a]	138-226	14	NP	05
CH05a05[a]	198-230	6	NP	06
CN445290[b]	230-242	3	218	06
NZ23g4[c]	84-116	9	NP	06
CN444794[b]	230-306	8	238	07
Z38126[b]	214-240	3	NP	07
Hi23g12[b]	223-241	5	NP	08
CH02b07[a]	180-202	7	NP	10
CH02a10[a]	143-177	6	135	10
CH03d11[a]	115-181	6	225	10
Hi16d02[b]	144-177	5	NP	11
CH02d08[a]	210-254	7	180	11
CH04a12[a]	158-196	8	140/184	11
Hi08f06[b]	224-242	5	NP	13
CH05f04[a]	160-172	6	NP	13
CH03a08[a]	146-218	7	148	13[d]
CH01g05[a]	140-188	6	NP	14
CH03a02[a]	124-184	9	NP	14
CH01e01[a]	106-120	5	NP	14[d]
CH05a02[a]	111-135	7	NP	15
CH04f10[a]	144-254	9	227	16
Hi02f12[b]	130-150	6	242	17
AY187627[b]	300-300	2	NP	17[d]
AF527800[b]	168-194	5	157/159	17

NP no products

[a] Liebhard et al. (2002)

[b] Silfverberg-Dilworth et al. (2006)

[c] Guilford et al. (1997)

[d] Same linkage group in loquat

Transferability and Polymorphism of Apple SSR to Loquat

The transferability of microsatellites among Rosaceae has been described (Dirlewanger et al. 2002; Wünsch and Hormaza 2007, Sargent et al. 2009) and molecular marker linkage maps of apple and *Prunus* have been shown to have a high level of macro-synteny (Dirlewanger et al. 2004). *Eriobotrya* has the same chromosome number ($2n=34$) as apple. Microsatellite markers used in loquat linkage mapping have been mainly derived from *Malus*, *Prunus*, and *Pyrus* (Soriano et al. 2005; Watanabe et al. 2008; Gisbert et al. 2009a) with only 21 polymorphic simple sequence repeats derived from an enriched loquat genomic library (Gisbert et al. 2008). The ratio of apple SSR that are polymorphic in loquat is not high, varying between 31% (14/45, Watanabe et al. 2008) and 55% (39/71, this research), with intermediate values (43%, 13/30) reported by Soriano et al. (2005).

The level of polymorphism of an SSR marker seems to greatly depend on the germplasm on which it is tested.

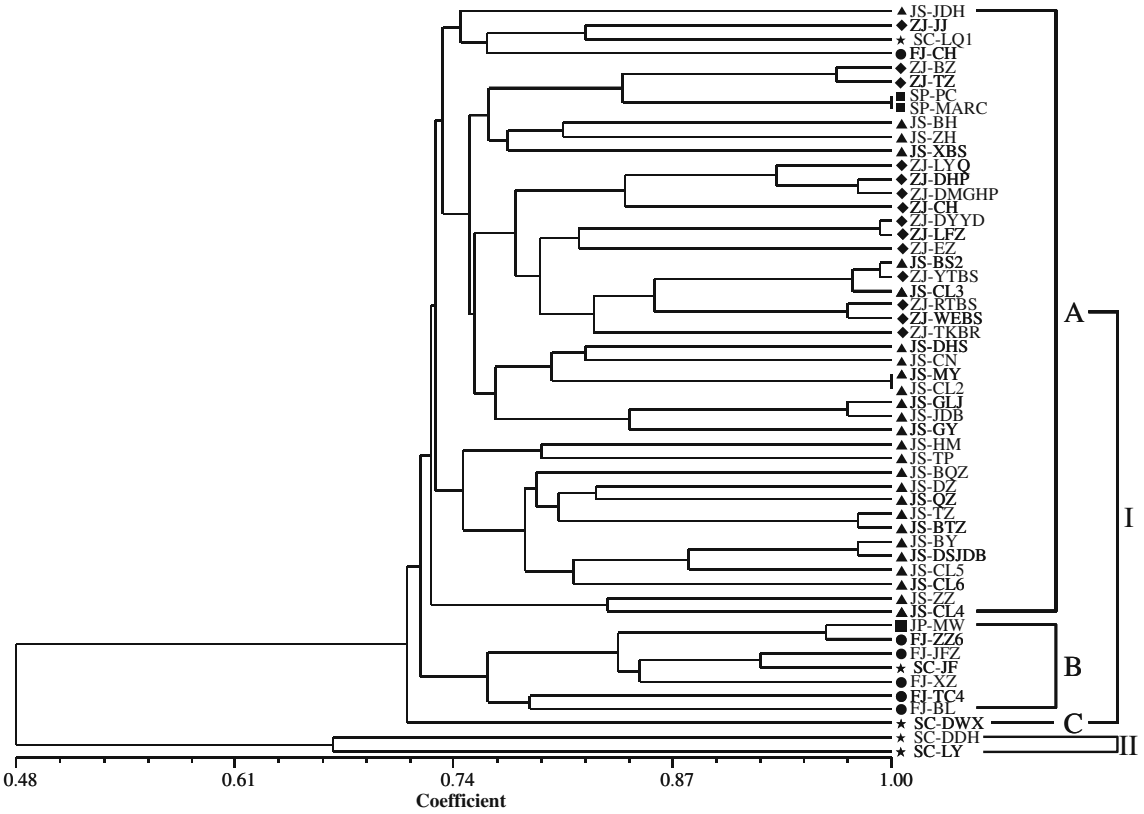

Fig. 3 Dendrogram of the 54 loquat accessions with 39 primer pairs generated by UPGMA cluster analysis from the similarity matrix obtained using simple matching (SM) coefficient; *filled square* indicates Japanese and Spanish accessions, *other symbols* as Fig. 1

Gisbert et al. (2009b) amplified 12 different alleles using Hi03a03 and distinguished most of their cultivars, whereas we only amplified three alleles with this SSR, as shown in Tables 4 and 5, the observed Ho we found was lower than that found by other authors. This is most likely due to the accessions used in this study being mostly seedling progenies or bud mutations of ancient cultivars with a narrow genetic background. More Chinese and European accessions are

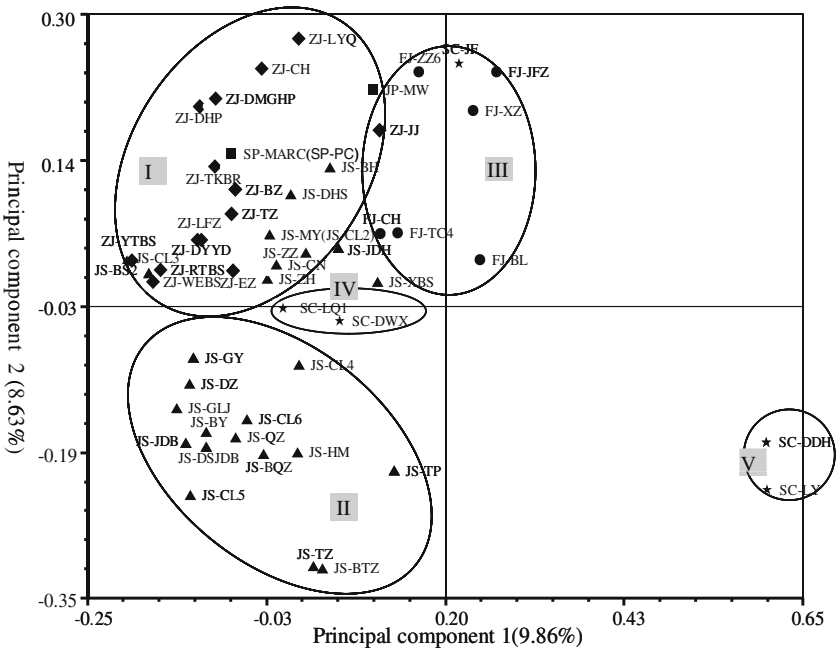

Fig. 4 Two-dimensional plot of the principal components analyzed in the 54 loquat accessions using SM coefficient

Table 4 Polymorphism comparison between this work and the literature

Na	He	Ho	Fis	Reference	Remark
1-6 (2.4)	0.27-0.65 (0.46)	0.21-0.73 (0. 51)	−0.98 to 0.65 (−0.20)	Soriano et al. 2005	13 SSR, 40 accessions
-[a]	0.17-0.81 (0.56)	0.20-1.00 (0.54)	-[a]	Gisbert et al. 2008	18 SSR, 21 cultivars
-[a]	(0.62)	-[a] (0.57)	-[a]	Gisbert et al. 2009b	5 SSR, 83 accessions
2-7 (3.4)	0.04-0.76 (0.50)	0.00–0.83 (0.47)	−0.70 to 0.87 (0.07)	This work	35 SSR, 52 accessions

[a] Data not shown; mean value in brackets

Table 5 The results found by Soriano et al. (2005) compared to this work (data in brackets)

Primer pair	locus	Size range (bp)	No. of alleles	He	Ho	*F*
CH01f02	CH01f02	170-180 (168-222)	3 (3)	0.33 (0.04)	0.49 (0.00)	−0.46 (1.00)
CH02f06	CH02f06	160-190 (118-121)	2 (2)	0.65 (0.50)	0.23 (0.50)	0.65 (0.01)
CH01f09	CH01f09	130-205 (112-139)	6 (2)	0.34 (0.50)	0.72 (0.56)	−1.12 (−0.12)
CH01h02	CH01h02	220-240 (222-241)	3 (4)	0.27 (-)	0.61 (-)	−1.27 (-)
CH02d12	CH02d12(1)	290-390	2 (-)	0.53 (-)	0.38 (-)	0.27 (-)
	CH02d12(2)	230-250 (221-242)	3 (5)	0.41 (0.76)	0.8 (0.75)	−0.98 (0.00)
CH02c06	CH02c06(1)	195-205 (160-190)	2 (2)	0.55 (0.45)	0.65 (0.56)	−0.19 (−0.25)
	CH02c06(2)	170	1	-	-	-

- Data not shown

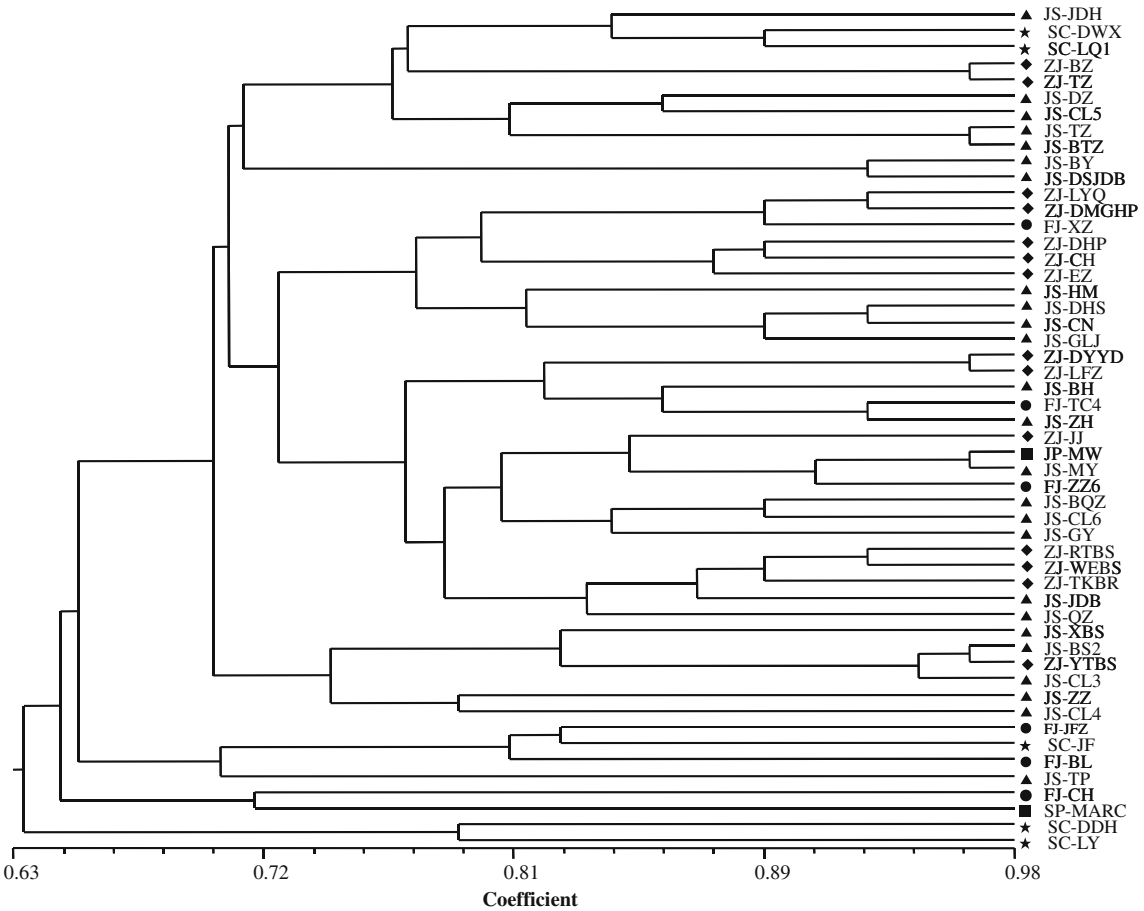

Fig. 5 Dendrogram of 52 of the loquat accessions (excluding the two sports) with five core primer pairs generated by UPGMA cluster analysis from the similarity matrix obtained using SM coefficient

needed, including wild species and semi-cultivated varieties, to reveal the total genetic diversity of loquat.

The size of the alleles and the number of loci has also been found to vary when using SSR markers. For example, with locus CH01f02 in loquat, Soriano et al. (2005) found three alleles between 170 and 180 bp long, and Gisbert et al. (2009b) found four alleles between 160 and 185 bp, whereas we found only two alleles of 160 and 162 bp. Another example is pear, where nine alleles between 155 and 180 bp long have been found in Tunisian pears (Brini et al. 2008), while eight alleles from 156 to 178 bp were found in European pears (Wünsch and Hormaza 2007). This may be caused by artificial deviation as a result of comparing the alleles with DNA markers.

Quality of Marker Scoring

In most diversity studies of loquat, the consistency of the marker scores cannot be checked. Errors in scoring remain unnoticed and lead to false levels of high diversity between accessions. As a control in our marker scoring system, we included some cultivars with known genetic relationships, such as 'Zaozhong No.6' and 'Changlv No.5', where, for each SSR locus, one allele of a progeny was present in each parent to give 100% scoring accuracy. We also compared our data with the literature to confirm the consistency of our scores: our scores on Moriowase were consistent with those of Watanabe et al. (2008) on Mogi, from which Moriowase is derived.

Grouping and Relationships of Accessions

Most of the new loquat cultivars are derived from natural hybridization (seeds) or bud sports, as *E. japonica* is both self-compatible and cross-compatible. Seedlings are usually quite heterogeneous and can easily be distinguished from each other, while accessions derived from mutation (and selected out by growers) cannot be distinguished. This is true for 'Meiyu' and 'Changlv No.2', with identical scores for each of our 39 SSR markers, and for the two Spanish cultivars 'Peluches' and 'Marc'. Our results are in agreement with those of Vilanova et al. (2001), who observed no difference with 23 polymorphic RAPD primers, and Soriano et al. (2005) who found a genetic distance so low (0.02) that it could be based on a single erroneous score of one of the 30 tested SSR markers. Peluches is thought to be a mutant of Algerie (Gisbert et al. 2009b), and it is probably also the case that Marc is derived from Algerie or one of its mutants.

Loquat was introduced to Japan from China in ancient times, and spread to Europe and other continents, so

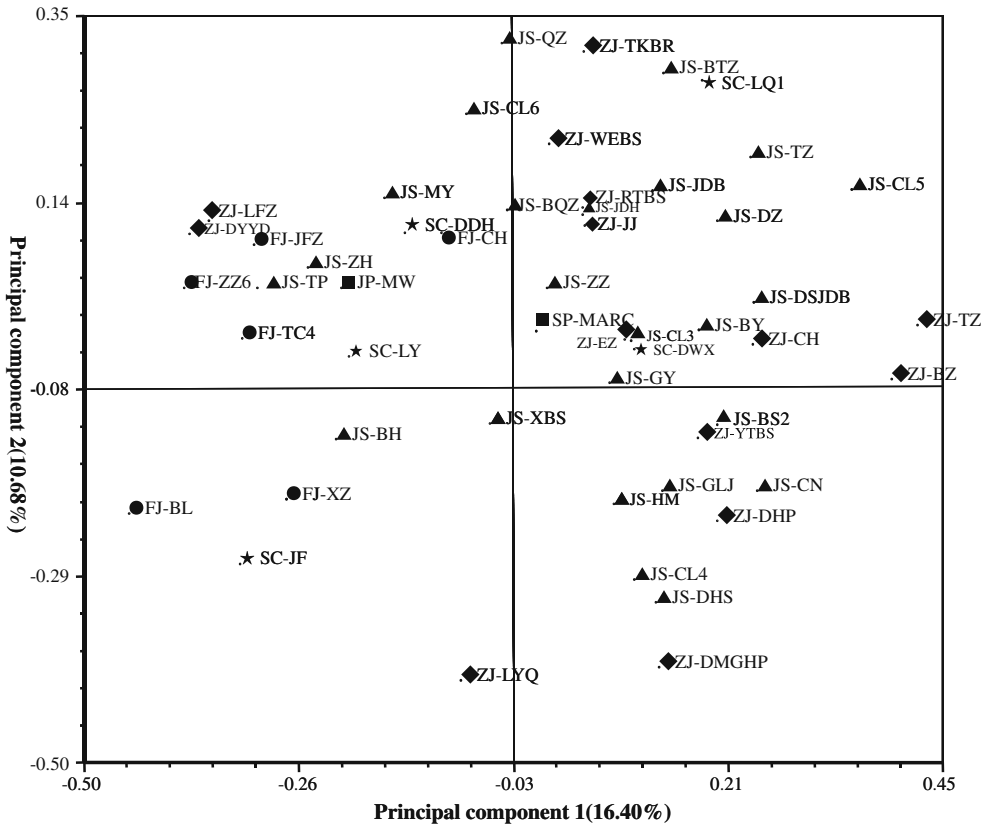

Fig. 6 Two dimensional plot of the principal components analyzed of 52 of the loquat accessions (excluding the two sports) with five core primer pairs, using the similarity matrix obtained with SM coefficient

Japanese loquat accessions are expected to have close relationship to Chinese accessions. The Spanish loquat accessions, 'Peluches' and 'Marc', clustered with Zhejiang and Jiangsu, which indicates that Spanish loquat was directly introduced from the Zhejiang or Jiangsu provinces. This is consistent with the results of Cai et al. (2003), using allozyme analysis, and Qiu and Zhang (1996). This is not surprising, as the Zhejiang province has been a major loquat producing area and famous for cultivars with good flavor quality since the Tang Dynasty (618-907 AD). The cluster analysis of the estimated genetic distance grouped the accessions according to their pedigrees and geographic origin. This result is similar to that found by Soriano et al. (2005).

Core set of Reference Accessions and Markers

A core set of highly divergent reference cultivars would be useful for evaluating the allelic diversity of new markers. For *Malus sieversii*, such a core collection was constructed based on 128 SSR alleles and 109 *M. sieversii* accessions. Accessions were selected by a preferred-allele sampling strategy combined with SM, Jaccard, and Nei & Li genetic distances, using a stepwise clustering approach (Zhang et al. 2009). Following the same procedure (with a threshold of 0.85 for SM coefficient), we obtained a core reference set of 20 loquat accessions based on our germplasm: 'Jidanhong', 'Luoyangqing', 'Dayeyangdun', 'Jiajiao', 'Bahong', 'Hongmao', 'Dahongsha', 'Taicheng No.4', 'Jiefangzhong', 'Changhong', 'Tongpi', 'Gaoliangjiang', 'Ruantiaobaisha', 'Biqizhong', 'Dazhong', 'Bingtangzhong', 'Guanyu', 'Dawuxing', 'Longquan No.1', and 'Marc'. Considering the literature (Yang et al. 2007; Tang 1997), it seems useful to extend this set with the two additional wild accessions: Daduhe and Liye.

To identify the maximum number of accessions with the minimum number of markers, we drew up a set of five core reference markers (CH03a09, CH02c06, CH04g12, CH02d12, and CH05h05) able to distinguish all our accessions except the sports (Figs. 5 and 6).

Linkage Mapping of Loquat

The first loquat linkage map was constructed with 'Algerie' and 'Zaozhong No. 6', which contained only 75% of the tested SSR markers, 25% of the markers being homozygous in at least one of the parents, and only five of their 111 SSRs (MS06g03, AU223657, CH03d07, CH04g12, and CH05h05) suitable for the 'Marc' x 'Zaozhong No. 6' cross (Gisbert et al. 2009b). Heterozygosity and similarity analyses of the tested cultivars could support the generation of a mapping population, with 'Daduhe' (*E. prinoides var. Daduheensis*) and 'Ruantiaobaisha' two candidate parents for crossing.

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