Microsatellite Markers and Genetic Diversity in Japanese Apricot (*Prunus mume*)

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Abstract. Sequencing amplification fragments produced using simple-sequence repeat (SSR) primer pairs pchgms2 and UDP96008 in ‘Dayezhugan’ japanese apricot showed that SSRs obtained included a microsatellite locus originally identified in peach. The microsatellite sequence homogeneity between UDP96008 in japanese apricot in this study and UDP96008 in the peach in GenBank was 98%. Twenty-four japanese apricot genotypes originating in diverse geographic areas had been identified with 14 SSR primer pairs developed in different species of *Prunus*. In total, 129 alleles were obtained and per primer pairs detected 2.5 alleles on the average. The results from cluster analysis showed that the genetic distance between ‘Nanhong’ and ‘Zhonghong’ was the closest, and cultivars from China and from Japan could not be separated completely.

Japanese apricot (*Prunus mume* Sieb. et Zucc.) originates in southern China and has been widely cultivated in China and Japan. It is used as fruit production and ornamental appreciation. Since most of the fresh *Prunus mume* fruit are sour, they are usually processed into different products, not for fresh-market sale, such as mume wine and candies. Several *Prunus mume* genotypes originating in China have been identified and classified with morphological characteristics (Gao et al., 1999a; Zhang et al., 1996) and isozyme analysis (Chu et al., 1988). However, these methods are limited with environmental influences and low levels of available polymorphism. The molecular markers, such as random fragment-length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPD) markers, amplified fragment-length polymorphisms (AFLPs), and simple-sequence repeats (SSRs), open a new approach to characterize and study the genetic relationship among different species and cultivars. The SSR marker is generally codominant and highly polymorphic, and can be detected with a simple polymerase chain reaction (PCR) procedure and can be found in large numbers covering the whole genome of the plant. Prior studies have used RAPD markers to analyze parentage determination, classification and self-compatibility of japanese apricot (Ozaki et al., 1994; Ozaki and Shimada, 1995; Tao et al., 2000).

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SSR marker is a powerful DNA marker to study molecular characterization of fruit trees (Gao et al., 2002b). Many studies on *Prunus* species demonstrate that primers developed in one species can amplify the microsatellite loci of some genotypes of other species (Cipriani et al., 1999; Downey and Iezzoni, 2000; Hormaza, 2002; Serrano et al., 2002). However, no application of SSR technique has been reported in japanese apricot, and no SSR primer pairs have been developed from japanese apricot so far.

In this study, we tried to use SSR primer

pairs previously developed in other *Prunus* species to identify japanese apricot genotypes originating from diverse geographical regions and further to analyze their genetic relationship.

Materials and Methods

Plant material. In total, 24 japanese apricot genotypes (19 from China, 5 from Japan) were used in this study (Table 1). A young leaf tissue sample of each genotype was collected from the japanese apricot orchards located at Nanjing Agricultural University, Nanjing, China.

DNA extraction. Extracted DNA was qualified in a 1.0% agarose gel to check its completeness and then quantified spectrophotometrically (GeneQuant, Pharmacia Biotech, Piscataway, N.J.), diluted to 60 ng·μL⁻¹ with TE buffer and used for PCR amplification.

DNA amplification and sequencing of the SSR fragments. Japanese apricot microsatellites were PCR-amplified as previously described (Gao et al., 2002b). PCR reactions were carried out in a PTC-100 thermocycler (MJ-Research, Watertown, Mass.) by an initial denaturation for 8 min at 94 °C followed by one cycle of 2 min at 94 °C, 50s at 61 °C, 50s at 72 °C; then 20 cycles of 40s at 94 °C, 50s at 61 °C, 50s at 72 °C; and then 20 cycles of 45s at 94 °C, 50s at 61 °C, 50s at 72 °C; and a final extension of 7 min at 72 °C. The PCR products were detected by a 8% nondenatured polyacrylamide gel electrophoresis in 1× TBE buffer. Gels were run for 5 h at 250 v. Following the electrophoresis, the gel was silver-stained according to the protocol described by Gao et al. (2002b). The sizes of DNA fragments were estimated with the 50-bp ladder-DNA sizing markers (MBI Fermentas).

The amplification products produced by two SSR primer pairs (pchgms2 and UDP96008) were sequenced and analyzed.

SSR of the cultivars and data analysis. Fourteen primer pairs as shown in Table 2

Table 1. List of the 24 japanese apricot cultivars used in this study and total number of DNA fragments.

Code of cultivars	Cultivars	Local origin	Avg fruit wt (g)	Total no. of bands
1	Zaohong	Zhejiang (China)	25.2	29
2	Zhonghong	Zhejiang (China)	19.7	39
3	Wanhong	Zhejiang (China)	15.6	35
4	Nanhong	Jiangsu (China)	15.6	38
5	Xiaoyezhugan	Zhejiang (China)	18.7	40
6	Dayezhugan	Zhejiang (China)	20.8	37
7	Jiuzhongmei	Zhejiang (China)	25.8	34
8	Longyanmei	Fujian (China)	15.4	39
9	Yeliqing	Zhejiang (China)	18.2	39
10	Henghe	Guangdong (China)	32.2	42
11	Dalizhong	Guangdong (China)	30.0	32
12	Zaohua	Guangdong (China)	10.0	41
13	Tonglv	Hunan (China)	12.3	39
14	Guangdonghuangpi	Guangdong (China)	12.6	40
15	Siyuemei	Hunan (China)	18.9	40
16	Sichuanhuangmei	Sichuan (China)	20.0	28
17	Taoxingmei	Guangdong (China)	18.2	31
18	Yunnanxingmei	Yunnan (China)	29.0	22
19	Qijiangxingmei	Sichuan (China)	22.4	32
20	Nankouume	Japan	17.8	40
21	Shirokaga	Japan	21.4	35
22	Hanakami	Japan	16.7	35
23	Koushiushinkou	Japan	4.5	32
24	Bungo	Japan	15.2	18

previously developed in peach and cherry (Cipriani et al., 1999; Sosinski et al., 2000) were used to identify the genetic diversity of 24 Japanese apricot germplasm. Amplified fragments were scored as present or absent. A similarity matrix was generated using the equation discussed in Nei and Li (1979) based on the proportion of the shared amplification fragments. Genetic relationships among the 24 genotypes included in this study were investigated using unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener, 1958) cluster analysis and depicted in a dendrogram. All analyses were computed with the SAS version 6.0 program.

Results

SSR analysis of Japanese apricot germplasm. Of the 24 SSR primer pairs screened, three primer pairs (PS7a2, UDP97401 and PS9f8) failed to amplify DNA bands in Japanese apricot. Seven primer pairs failed to amplify DNA bands in all cultivars (UDP96015 and UDP97403) or the products were smeared (UDP96013, UDP97402, and UDP98406) or not repeatable (UDP98408 and Ps12e2). In total, 14 primer pairs were used in the analysis. From those primer pairs, 11 have been developed in peach, 1 in sour cherry and 2 in sweet cherry. The 14 primer pairs produced 129 alleles with a mean value of 2.5 alleles per locus. The size of the amplified fragments was consistent with that of expected from other species as shown in Table 2. The number of amplification bands per genotype was from 18 to 42, and the average value was 35 as shown in Table 1. More than one polymorphic fragments were produced by all primer pairs except the primer pair PS8e8. Amplification patterns with primer pairs UDP98405, UDP96008, UDP98409 and Dpchgms3 were as shown in Fig. 1. The frequency of DNA polymorphisms by SSR assay was sufficiently high to survey the phylogeny of *Prunus mume*. Six cultivars (Nos. 1, 5, 8, 9, 11, 17) could be identified with the primer pairs UDP98409 as shown in Fig. 1C. Five cultivars (Nos. 8, 11, 13, 14, 23) could be identified with the primer pairs UDP98405 as shown in Fig. 1A, and no unique SSR primer pair could identify all cultivars.

The dendrogram was obtained after UPGMA cluster analysis of the similarity data as shown in Fig. 2. Genetic distance values between cultivars ranged from 0.39 to 1.10. These cultivars were divided into two groups at 1.1 genetic distance. The value of genetic distance between cultivars 'Nanhong' and 'Zhonghong' was the smallest, which suggested that the genetic relationship of these two cultivars was the closest. The dendrogram also showed that cultivars from China and from Japan could not be separated completely. Five cultivars from China ('Zaohong', 'Xiaoye-

Fig. 1. SSR banding patterns of 24 Japanese apricot cultivars in polyacrylamide gel separation with different primer pairs: A UDP98405 B UDP96008 C UDP98409 D Dpchgms3. Lanes corresponding to the codes of the cultivars in Table 1. M = molecular marker (50-bp ladder).

Table 2. List of SSR markers that produced polymorphic fragments among 24 Japanese apricot cultivars.

Locus code	Origin	Primer sequences (5' to 3')	Predicted length(bp)	Size range (bp)	No. of alleles
pchgms1	Peach	GGGTAAATATGCCCATTTGTGCAATC GGATCATTGAACTACGTCAATCCTC	194	120–200	18
pchgms2	Peach	GTCAATGAGTTTCAGTGTCTACACTC AATCATAACATCATTACGCCACTGC	163	130–200	14
pchgms3	Peach	ACGGTATGTCCGTACACTCTCCATG CAACCTGTGATTGCTCCTATTAAC	179	145–220	10
UDP96003	Peach	CCCAATGAACAACCTGCAT CATATCAATCACTGGGATG	143	90–150	15
UDP96005	Peach	GTAACGCTCGCTACCACAAA CCTGCATATCACCACCCAG	155	100–250	5
UDP96008	Sweet cherry	TTGTACACACCCTCAGCCTG TGCTGAGGTTTCAGGTGAGTG	165	140–160	10
UDP96010	Peach	CCCATGTGTGTCCACATCTC TTGATGATTCCATGCGTCTC	131	50–200	7
UDP96018	Peach	TTCTAATCTGGGCTATGGCG GAAGTTCACATTTACGACAGGG	253	250–270	3
UDP96019	Peach	TTGGTCATGAGCTAAGAAAACA TAGTGGCACAGAGCAACACC	216	170–240	8
UDP98405	Peach	ACGTGATGAACGTACACCCA GAGTCTTTGCTCTGCCATCC	104	100–150	7
UDP98407	Peach	AGCGGCAGGCTAAATATCAA AATCGCCGATCAAAGCAAC	212	80–250	11
UDP98409	Peach	GCTGATGGGTTTTATGGTTTTTC CGGACTCTTATCCTCTATCAACA	129	125–165	16
PceGA34	Sour cherry	GAACATGTGGTGTGCTGGTT TCCACTAGGAGGTGCAAATG	155	135–170	3
PS8e8	Sweet cherry	CCCAATGAACAACCTGCAT CATATCAATCACTGGGATG	183	180	1
UDP96013	Peach	ATTCTTCACTACACGTGCACG CCCAGACATACTGTGGCTT	198	100–250	---
UDP96015	Peach	CCTTGACCTATTTGTTTCGTC ACTAGTCAAACAATCCCCCG	174	30–400	---
UDP97401	Peach	TAAGAGGATCATTTTTGCCTTG CCCTGGAGGACTGAGGGT	130	ND ^a	---
UDP97402	Peach	TCCCATAACCAAAAAAACACC TGGAGAAGGGTGGGTACTCA	136	70–150	---
UDP97403	Peach	CTGGCTTACAACCTCGAAGC CGTCGACCAACTGAGACTCA	150	30–150	---
UDP98408	Peach	ACAGGCTTGTGAGCATGTG CCCTCGTGGGAAAATTGA	100	90–200	----
PS7a2	Sweet cherry	CAGGGAAATAGATAAGATG TCTAATGGTGGTGTTCATT	164	ND ^a	---
PS9f8	Sweet cherry	GGTTCCTGGTTATTATGA ACATTCTATGCAGAGAT	156	ND	---
PS12e2	Sweet cherry	GCCACCAATGGTTCTTCC AGCACCAGATGCACCTGA	164	50–200	---
UDP98406	Peach	TCGGAAACTGGTAGAAGACA ATGGGTCGTATGCACAGTCA	101	30–100	---

^aNo data.

Fig. 2. Dendrogram of 24 Japanese apricot cultivars based on UPGMA analysis using the similarity matrix generated by the Nei and Li coefficient after amplification with 14 pairs of microsatellite primers.

(1)TTGTACACAC CCTCAGCCTG TCACGCAAAA AGAAATAATA AGCAAGGGAG CA

(2)TTGTACACAC CCTCAGCCTG TCACGCAA AA AGAAATAATA AGCAAGGGAG CA

(1)CCCAGGCA GGCATGATAA CACCTACACA CACACACACAGAG....AAA CAGA

(2)CCCAGGCA GGCATGATAA CACCTACACA CA CACACACA CA GAGAG AAA CA GA

(1)GATAGTCAAC AACACTCACC TGAACCTCAG CA

(2)GATAGT CAACAACACT CACCTGAA CC TCAGCA

Fig. 3. Sequence alignment of UDP96008 SSR marker in different *Prunus* species. 1) sequence of UDP96008 from sweet cherry (GenBank accession No. AF501740). 2) sequence of PCR product from 'Dayezhugan' japanese apricot with UDP96008 primer pairs.

zhugan', 'Dayezhugan', 'Jiuzhongmei', 'Guangdonghuangpi') and three cultivars from Japan ('Nankoume', 'Hanakami', 'Koushi-ushinkou') were in one group at 0.82 genetic distance. Another group included two cultivars from China ('Tonglv', 'Siyuemei' and one cultivar from Japan 'Shirokaga').

Sequences of PCR products with SSR primer pairs pchgms2 and UDP96008. The sequence of PCR products in japanese apricot cultivar Dayezhugan with SSR primer pairs pchgms2 was 138 base pairs, and the simple sequence repeat was (AG)₁₀, the following was the sequence of the microsatellite locus: CAGT-TACTCA AGTCACAGAT GTGAGTTTTTCTTGAACATTC TGAGTTTTTGA ACTAT-CAGCCGAGATCAGAG AGAGAGAGAG AGAGAGAAGG GGGTTTTTTTA ATGTA-ACGAT AAAAATCATA ACATCATTTCA GCCACTGC (GenBank accession No. AY216737). The simple sequence repeat from peach is (AG)₂₄.

As for primer pair UDP96008, the simple sequence repeat obtained in japanese apricot was (CA)₈, whereas the simple sequence repeat obtained in sweet cherry was reported as (CA)₇ (GenBank accession No. AF501740). The sequence homogeneity between the two microsatellites was 98% as shown in Fig. 3.

Discussion

Sharing of SSR primer pairs between different species. Widespread use of SSR markers is often limited by the cost of developing of the primer pairs since it involves library generation and sequencing. Many studies indicate that microsatellite sequences are highly conserved in *Prunus* (Hormaza, 2002; Serrano et al., 2002). Recently SSR primer pairs have been developed from *Prunus* species, mostly peach (Aranzana et al., 2002; Cipriani et al., 1999; Dirlewanger et al., 2002; Sosinski et al., 2000; Testolin et al., 2000), sweet cherry, sour cherry (Downey and Iezzoni, 2000), and apricot (Lopes et al., 2002). Though there are no SSR primers in japanese apricot, other *Prunus* SSR primer pairs could be used to identify japanese apricot germplasm. In this

study, SSR amplification products in japanese apricot were sequenced and compared those obtained from sweet cherry. The result showed that the sequences obtained contained simple sequence repeats locus and the number of sequence repeats was different in different species. This study also demonstrated that SSR primer pairs developed in peach, sweet cherry and sour cherry could be shared in identification of japanese apricot germplasm.

Relationship among japanese apricot genotypes. Many researchers reported that *Prunus mume* is native to China and *Prunus mume* cultivars in Japan are introduced from China (Yoshida et al., 1999). In this study, cultivars from Japan could not be separated by SSR assay from cultivars from China, which suggested that there were no clear genome differences between Chinese cultivars and Japanese cultivars.

The genetic distance between cultivars 'Nanhong' and 'Zhonghong' was the closest from the dendrogram, which indicated that these two cultivars may have the same origin. The cluster results from SSR markers were in agreement with the results from morphological characteristics (Zhang et al., 1996; Gao et al., 1999a), which suggested that SSR marker could be used to study the genetic relationship of *Prunus mume*.

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