A Practical Method for Peach-related Species Identification and Hybrid Analysis Using Simple Sequence Repeat Markers

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ABSTRACT. Cultivated peach (*Prunus persica*) is an important fruit species worldwide. The wild relatives in *Prunus*, such as *P. mira*, *P. davidiana*, *P. kansuensis*, *P. ferganensis*, and *P. persica*, are valuable for peach breeding, and early and accurate identification of parental and hybrid genotypes is critical. In this study, 20 representative accessions of peach germplasm from the National Germplasm Repository of Peach in China were used to select a set of 18 simple sequence repeat (SSR) markers for accurate species discrimination. Eight unknown peach samples were successfully identified using the SSR panel and species genotype database. Interspecific hybrid genotypes of *P. persica* × *P. davidiana*, *P. persica* × *P. kansuensis*, and *P. persica* × *P. ferganensis* were also analyzed reliably. The markers were amenable to high-throughput fluorescent labeling and capillary electrophoresis (CE) analysis, allowing rapid and efficient species identification. The practical method described in this study will facilitate peach breeding and germplasm management.

Peach is one of the most important fruit species worldwide. China, where peach originated, has the richest resource of species related to cultivated peach. These species contributed to peach domestication history over more than 4000 years, with some cultivars disseminating to Central Asia, the Mediterranean coast, Europe, America, and Japan (Layne and Bassi, 2008; Wang and Zhuang, 2001). The main relatives of cultivated peach, namely *P. mira*, *P. davidiana*, *P. kansuensis*, and *P. ferganensis*, are found readily in southwestern and northwestern China. Fruit from almost all of the related wild species exhibit poor eating quality, but these species could be valuable as sources of pest and disease resistance traits or as rootstock (Cao et al., 2011; Moing et al., 2003). Increasing numbers of related species are being used to develop new peach cultivars. Efficient and accurate species identification and hybrid analysis are necessary in peach breeding and germplasm management.

A range of practices were used previously to identify peach-related germplasm, including morphology (Wang and Zhuang, 2001), cytology (Guo et al., 1996), palynology (Wang and Zhou, 1990), isozymes (Mowrey et al., 1990), and DNA markers (Cheng et al., 2001; Yu et al., 2004). With the development of sequencing technology, peach genome sequences have become available (Verde et al., 2013), and high-throughput single-

nucleotide polymorphism (SNP) tools have been used for large-scale genetic analysis in peach cultivars and accessions (Cao et al., 2014; Verde et al., 2012).

SSR (microsatellites) are tandem repeat DNA sequences with a core unit of 1–6 bps, which are abundant in prokaryotic and eukaryotic genomes and are ubiquitously distributed in both the protein-coding and noncoding regions (Guichoux et al., 2011; Kalia et al., 2011). The high variability of microsatellites usually manifests as different numbers of repeats in the region of the repeated motif, and short insertion/deletion events are also seen (Decroocq et al., 2003). SSR markers are ideal for profiling as they are codominant and can display a large number of alleles per locus. In addition, assaying SSR markers is relatively simple and reproducible and is accomplished using polymerase chain reaction (PCR)-based methods that are amenable to automation (Kalia et al., 2011). SSR markers proved to be highly efficient for genetic analysis in several fruit species, such as grape [*Vitis vinifera* (Emanuelli et al., 2013)], jujube [*Ziziphus jujuba* (Ma et al., 2012)], almond [*Prunus dulcis* (Dangl et al., 2009)], and sweet cherry [*Prunus avium* (Lacis et al., 2009)]. SSR markers were developed previously in peach and used for genetic diversity assessment (Li et al., 2008), cultivar identification (Chen et al., 2011; Li et al., 2013), trait mapping (Lambert and Pascal, 2011; Liu et al., 2009), and phylogenetic studies (Cheng and Huang, 2009). However, to date, reliable and efficient SSRs for discrimination of peach-related species have not been developed, and no database of species genotypes has been established. A universal database of SSR marker profiles from peach-related species would facilitate peach breeding and germplasm management.

The NGRPC, located in Zhengzhou (central China), Nanjing (eastern China), and Beijing (northern China), is the most

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important germplasm repository of peach, nectarine, and related species in China. The NGRPC preserves original and representative accessions of species related to peach and is the core collection of such species for China and the world. Here, we describe a practical SSR-based method for discriminating species related to peach and for identifying interspecific hybrids. The aims of this study were to 1) screen SSRs and develop a set of markers for efficient identification of peach-related species, 2) establish a universal genotype database for peach-related species using fluorescent-labeled SSR markers, and 3) demonstrate the practical utility of the SSR set for unknown genotype identification and interspecific hybrid analysis.

Materials and Methods

PLANT MATERIAL. Twenty-nine *Prunus* samples were used in the study (Table 1). Among them, 20 samples (codes 1–20) were obtained from three NGRPCs (Zhengzhou, Nanjing, and Beijing). These 20 accessions were representative samples of five species (*P. persica*, *P. davidiana*, *P. kansuensis*, *P. ferganensis*, and *P. mira*) that were established on the core collection during initial investigations in China in the last century. Some of these representative accessions were described using classical morphological characteristics, regional origin, and landrace names. Eight samples (codes 21–28) were unknown accessions awaiting species identification, and one sample (code 29) was a cultivar that was used as a maternal parent for hybridization. Three interspecific hybridizations were performed by the Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences (ZFRI, CAAS), as follows: *P. persica* (code 29) × *P. davidiana* (code 1), *P. persica* (code 29) × *P. kansuensis* (code 5), and *P. persica* (code 29) × *P. ferganensis* (code 10). Three F₁ hybrid populations were obtained and used alongside their parents for hybrid analysis.

DNA EXTRACTION. Fresh young leaves were collected and rapidly dried at room temperature using solid silica gel pellet desiccant. Genomic DNA was isolated using the modified cetyltrimethylammonium bromide protocol described by Cheng et al. (1997). DNA was quantified using a spectrophotometer (BioPhotometer plus; Eppendorf AG, Hamburg, Germany), and diluted to 20 ng·μL⁻¹ as template for PCR amplification.

SSR MARKERS. Seventy-eight SSR markers (Table 2), previously developed for peach (Aranzana et al., 2002; Dirlewanger et al., 2002; Sosinski et al., 2000; Testolin et al., 2000; Yamamoto et al., 2002), Japanese plum [*Prunus salicina* (Mnejja et al., 2004)], and almond (Mnejja et al., 2005), were tested for their ability to produce amplification products in *Prunus* species. The 78 loci were distributed across the whole *Prunus* genome, with 9–10 loci per linkage group.

PCR AMPLIFICATION. PCR amplification was performed in 20-μL reaction volumes containing 20 ng of genomic DNA, 10 μL of 2× Taq PCR MasterMix (Tiangen Biotech, Beijing, China), and 0.25 μM SSR primers. Amplification parameters were as follows: 5-min initial denaturation of the template DNA at 94 °C; 35 cycles of 45-s template DNA denaturation at 94 °C, 45-s primer annealing at the appropriate temperature, 45-s primer extension at 72 °C; and a final primer extension at 72 °C for 10 min. PCR products were stored at 4 °C before analysis by electrophoresis. SSR reactions were repeated for each SSR/DNA

Table 1. Species, sources, and characteristics of the 29 peach-related samples in *Prunus*.

Code ^z	<i>Prunus</i> species	Source ^y	Characteristic
1	<i>P. davidiana</i>	NGRPC, Zhengzhou	Red flower
2	<i>P. davidiana</i>	NGRPC, Zhengzhou	White flower
3	<i>P. davidiana</i>	NGRPC, Nanjing	Red flower
4	<i>P. davidiana</i>	NGRPC, Beijing	White flower
5	<i>P. kansuensis</i>	NGRPC, Zhengzhou	Red root
6	<i>P. kansuensis</i>	NGRPC, Zhengzhou	White root
7	<i>P. kansuensis</i>	NGRPC, Nanjing	
8	<i>P. kansuensis</i>	NGRPC, Beijing	Red root
9	<i>P. ferganensis</i>	NGRPC, Zhengzhou	Kashi no.1
10	<i>P. ferganensis</i>	NGRPC, Zhengzhou	Kashi no.2
11	<i>P. ferganensis</i>	NGRPC, Nanjing	Datianren
12	<i>P. ferganensis</i>	NGRPC, Beijing	
13	<i>P. mira</i>	NGRPC, Zhengzhou	Linzi, Tibet, China
14	<i>P. mira</i>	NGRPC, Zhengzhou	Aba, Sichuan, China
15	<i>P. mira</i>	NGRPC, Nanjing	
16	<i>P. mira</i>	NGRPC, Beijing	
17	<i>P. persica</i>	NGRPC, Beijing	Hubei, China
18	<i>P. persica</i>	NGRPC, Beijing	Guizhou, China
19	<i>P. persica</i>	NGRPC, Beijing	Sichuan, China
20	<i>P. persica</i>	NGRPC, Beijing	Hebei, China
21	unknown	ZFRI, CAAS	Chance seedling
22	unknown	ZFRI, CAAS	Chance seedling
23	unknown	ZFRI, CAAS	Chance seedling
24	unknown	ZFRI, CAAS	Chance seedling
25	unknown	ZFRI, CAAS	Chance seedling
26	unknown	ZFRI, CAAS	Chance seedling
27	unknown	ZFRI, CAAS	Chance seedling
28	unknown	ZFRI, CAAS	Chance seedling
29	<i>P. persica</i>	ZFRI, CAAS	Cultivar (Dajubao)

^zTwenty-nine samples including 20 representative accessions of five species (codes 1–20) in *Prunus*, eight unknown samples awaiting species identification (codes 21–28), and one cultivar (code 29).

^yNational Germplasm Repository of Peach in China (NGRPC), Zhengzhou Fruit Research Institute (ZFRI), Chinese Academy of Agricultural Sciences CAAS).

sample combination at least twice to identify an appropriate annealing temperature and ensure consistent results.

DATA ANALYSIS. The 78 PCR products of 20 representative accessions were analyzed using electrophoresis with 9% polyacrylamide gels. Gels were silver stained according to the protocol described by Bassam et al. (1991). Optimal SSR primers for species identification were selected according to their consistent and reliable amplification patterns and interspecific discrimination power. Subsequent analysis was performed with fluorescent-labeled primers and CE. The selected SSR primers were labeled with fluorescein on the top of each forward primer (5'-FAM), and the PCR products of 29 samples were separated by CE and detected using a DNA analyzer (ABI 3730XL; Genewiz Biotechnology, Beijing, China). All the alleles of each locus were ranked in size from small to large, and a binary data matrix was established based on allele presence (1) or absence (0) at each locus for each sample. A dendrogram was constructed to represent the genetic relationships between the 29 individuals according to their similarity coefficients. The dendrogram was constructed using the unweighted pair group method with arithmetic means (UPGMA) in NTSYSpc software (version 2.10e; Applied Biostatistics, East Setauket, NY).

Table 2. Simple sequence repeat (SSR) marker name and linkage group of the 78 SSRs used for peach related species identification.^z

SSR marker	Linkage group	SSR marker	Linkage group	SSR marker	Linkage group	SSR marker	Linkage group
CPPCT27	1	UDP98-025	2	BPPCT007	3	pchgms2	4
UDP96-005	1	BPPCT001	2	UDP97-403	3	<i>CPPCT5</i>	4
CPPCT26	1	UDP96-013	2	CPPCT18	3	UDP96-003	4
BPPCT020	1	pchgms1	2	BPPCT039	3	BPPCT015	4
CPPCT29	1	BPPCT034	2	CPPCT2	3	BPPCT023	4
BPPCT028	1	UDP98-406	2	UDP96-008	3	BPPCT031	4
BPPCT016	1	BPPCT013	2	BPPCT021	3	BPPCT010	4
pchcms4	1	BPPCT030	2	CPDCT008	3	CPPCT28	4
pchgms3	1	CPPCT24	2	CPDCT025	3	pchgms5	4
CPPCT16	1	<i>CPPCT5</i>	2	CPDCT027	3	<i>CPPCT3</i>	4
UDP97-401	5	CPPCT8	6	CPPCT22	7	BPPCT006	8
BPPCT017	5	UDP96-001	6	UDP98-405	7	BPPCT019	8
BPPCT037	5	BPPCT008	6	UDP98-408	7	UDP96-019	8
BPPCT038	5	BPPCT025	6	CPPCT33	7	CPPCT6	8
BPPCT032	5	UDP98-412	6	CPPCT17	7	UDP98-409	8
BPPCT014	5	UDP98-407	6	BPPCT029	7	CPPCT35	8
CPPCT9	5	pchcms5	6	<i>CPPCT3</i>	7	CPPCT19	8
CPPCT4	5	CPPCT15	6	pchcms2	7	BPPCT033	8
BPPCT026	5	CPPCT23	6	CPSCT004	7	MA013a	8
pchgms4	5	CPPCT30	6	CPSCT026	7	MA017a	8

^zTen representative loci were initially proposed in each *Prunus* linkage group based on the SSRs set proposed by Aranzana et al. (2003). A total of 78 SSRs were finally selected as candidates, as CPPCT5 and CPPCT3 occurred in two different linkage groups (highlighted in italics).

Table 3. The reasons for elimination of 60 simple sequence repeat (SSR) markers removed from further testing during prescreening on the 20 representative peach-related species accessions.

Elimination reason	SSR marker	SSRs (no.)
No amplification	CPPCT17, CPPCT18, CPPCT29	3
Random amplification	BPPCT020, CPPCT35, UDP96-003	3
Poor amplification	CPPCT27, BPPCT038, UDP96-008	3
Monomorphic	BPPCT029, CPPCT8, CPPCT9, CPPCT23, CPSCT004, CPSCT026	6
High intraspecific polymorphism	BPPCT007, BPPCT008, BPPCT015, BPPCT030, BPPCT039, MA017a, UDP96-019, UDP98-025, UDP98-409	9
Low interspecific polymorphism	BPPCT006, BPPCT013, BPPCT014, BPPCT016, BPPCT017, BPPCT019, BPPCT021, BPPCT026, BPPCT028, BPPCT031, BPPCT033, BPPCT037, CPDCT008, CPDCT025, CPDCT027, CPPCT2, CPPCT3, CPPCT4, CPPCT5, CPPCT6, CPPCT15, CPPCT19, CPPCT22, CPPCT24, CPPCT26, CPPCT28, MA013a, pchcms2, pchcms4, pchcms5, pchgms5, UDP97-403, UDP98-405, UDP98-406, UDP98-407, UDP98-408	36

Fig. 1. Separation of amplification products generated by simple sequence repeat (SSR) primer (A) CPSCT026, (B) UDP98-025, and (C) CPPCT30 in 20 representative accessions in *Prunus*. M = DNA marker, CK = blank control with double-distilled H₂O replacing DNA template, codes 1–20 = 20 representative accessions belonging to five species (*P. davidiana*, *P. kansuensis*, *P. ferganensis*, *P. mira*, and *P. persica*).

Results

SSR AMPLIFICATION AND SELECTION. The diversity of the 78 SSRs was assessed among the 20 representative peach samples of five species (*P. persica*, *P. davidiana*, *P. kansuensis*, *P. ferganensis*, and *P. mira*) from NGRPC. Sixty loci were eliminated from further analysis for the following reasons (Table 3). Alleles could not be scored for nine primer pairs: three primer pairs failed to amplify fragments, three amplified more than two random fragments, and three loci were difficult to score due to poor amplification. Alleles at an additional 51 loci could be scored but were not sufficiently discriminatory. Of these, six loci were monomorphic for all 20 accessions in the electropherogram (Fig. 1A); 36 loci exhibited low interspecific polymorphism, usually due to some very high-frequency alleles; and nine loci exhibited high intraspecific polymorphism (Fig. 1B). Eighteen loci were selected for further testing. All of the 18 loci were consistently amplifiable, had only one or two alleles at a locus in every specific accession, and exhibited high interspecific polymorphism and low intraspecific polymorphism (Fig. 1C).

Table 4. Allele sizes for 29 peach-related samples based on the 18 selected simple sequence repeat markers.

Code	Allele sizes (bp)																		
	BPPCT	CPPCT	BPPCT	UDP96-	UDP96-	pchgms	CPPCT	UDP97-	CPPCT	BPPCT	pchgms1	UDP96-	pchgms4	UDP96-	UDP98-	BPPCT	pchgms3	BPPCT	BPPCT
	010	30	032	005	013	2	33	401	16	001	pchgms1	001	pchgms4	001	412	034	pchgms3	025	023
1	111/121	171/175	175/175	134/156	149/149	155/171	142/142	110/110	199/199	132/132	208/208	141/155	108/108	98/108	108/108	205/228	186/186	147/147	211/227
2	111/121	175/175	183/173	138/154	151/151	155/171	146/146	106/110	195/195	132/132	194/208	141/173	139/139	115/121	115/121	205/240	186/186	171/185	229/229
3	121/129	165/169	173/173	138/164	151/151	145/161	144/144	106/106	175/175	132/132	194/194	141/141	108/108	94/117	94/117	236/236	182/194	147/179	211/225
4	111/111	165/169	181/181	158/175	149/149	145/151	148/148	100/106	197/197	132/132	208/208	141/141	110/110	94/106	94/106	213/213	186/186	153/153	179/215
5	139/139	221/221	190/190	132/132	212/212	185/185	161/161	98/98	179/179	130/130	200/200	169/169	106/106	113/113	113/113	207/207	175/175	183/183	215/215
6	139/139	221/221	190/190	130/130	212/212	185/185	161/161	98/98	179/179	130/130	200/200	165/165	106/106	113/113	113/113	207/207	175/175	183/183	215/215
7	139/139	221/221	190/190	132/132	212/212	185/185	161/161	98/98	179/179	130/130	200/200	169/169	106/106	113/113	113/113	207/207	175/175	183/183	215/215
8	141/141	221/221	190/190	130/130	212/212	181/181	161/161	98/98	179/179	130/130	200/200	169/169	106/106	113/113	113/113	207/207	175/169	185/185	215/215
9	131/131	191/191	200/200	175/175	179/179	155/155	152/152	123/123	193/193	160/160	191/191	173/173	125/125	125/125	125/125	238/238	204/204	175/175	181/181
10	131/131	191/191	200/200	164/164	179/179	155/155	152/152	121/121	193/193	154/154	191/191	173/173	125/125	125/125	125/125	238/238	206/206	175/175	181/181
11	131/131	191/191	200/200	175/175	179/179	155/155	152/152	123/123	193/193	154/154	191/191	173/173	125/125	125/125	125/125	226/226	204/204	175/175	181/181
12	131/131	193/193	200/200	158/158	179/179	155/155	152/152	123/123	193/193	154/154	191/191	173/173	125/125	125/125	125/125	226/226	204/204	175/175	181/181
13	117/117	151/151	163/163	122/122	261/261	126/126	137/137	121/121	183/183	146/146	183/183	150/150	96/96	86/86	86/86	224/246	198/198	181/181	215/215
14	117/117	151/151	163/163	122/122	226/226	126/126	137/137	121/121	183/183	146/146	183/183	150/150	104/104	86/86	86/86	224/246	200/200	193/193	215/215
15	117/117	151/191	163/163	122/122	179/179	126/126	137/137	123/123	183/183	152/152	191/191	165/173	86/86	86/86	86/86	238/238	204/204	191/177	181/181
16	117/117	151/151	163/63	122/122	224/224	126/126	137/137	172/172	183/183	152/152	183/183	150/150	86/96	86/96	92/92	224/246	206/206	175/181	181/181
17	127/127	193/193	200/194	156/156	163/163	155/155	148/148	121/121	193/193	152/152	191/191	173/173	119/119	119/119	119/125	224/238	175/175	189/193	181/181
18	127/127	193/193	200/200	156/156	179/179	155/155	152/152	121/121	193/193	152/152	191/191	175/175	117/117	117/117	119/119	224/238	175/175	191/191	181/181
19	127/127	191/191	202/202	156/156	179/179	155/155	150/150	119/119	205/205	162/162	193/193	173/173	125/125	113/125	113/125	238/238	182/204	191/191	181/181
20	127/127	191/191	200/200	173/173	163/163	157/157	142/142	121/121	185/185	156/156	191/191	173/173	125/125	113/127	113/127	238/238	175/190	171/187	211/211
21	121/129	167/175	204/181	154/166	179/179	165/171	144/144	110/110	187/187	134/134	194/204	141/141	137/137	100/121	100/121	205/221	188/188	171/185	209/209
22	121/131	165/167	181/181	128/136	153/153	153/153	142/142	106/106	199/199	134/134	194/202	141/141	108/108	94/113	94/113	221/240	188/188	147/177	215/215
23	121/129	165/175	173/273	130/130	167/167	153/153	142/142	110/110	181/181	134/134	208/208	141/157	108/108	94/119	94/119	209/242	182/182	157/157	211/211
24	131/131	193/193	200/200	158/158	179/179	155/155	152/152	123/123	193/193	152/152	191/191	173/173	125/125	125/125	125/125	238/238	204/204	175/175	181/181
25	131/131	193/193	200/200	158/158	179/179	155/155	152/152	123/123	193/193	154/154	191/191	173/173	125/125	125/125	125/125	226/226	204/204	175/175	181/181
26	131/131	191/191	200/200	175/175	179/179	155/155	152/152	121/121	193/193	152/152	191/191	173/173	125/125	125/125	125/125	224/224	204/204	175/175	181/181
27	127/127	171/171	200/200	175/175	167/167	155/155	148/148	121/121	189/189	156/156	191/191	173/173	113/121	121/121	121/121	226/226	202/202	175/193	181/181
28	127/127	193/193	206/194	156/156	163/163	155/155	148/148	121/121	193/193	142/152	193/193	177/177	121/121	121/121	121/121	213/236	175/175	175/181	181/211
29	127/127	191/191	200/200	158/173	163/163	155/155	148/148	121/127	191/191	152/156	191/191	171/177	117/125	127/127	127/127	224/238	175/175	187/193	211/221

Table 5. Simple sequence repeat (SSR) genotype database for five peach-related species in *Prunus* based on 18 SSR markers and 20 accessions.

SSR marker	Allele sizes (bp)				
	<i>P. davidiana</i>	<i>P. kansuensis</i>	<i>P. ferganensis</i>	<i>P. mira</i>	<i>P. persica</i>
BPPCT010	111/121/129	139/141	131	117	127
CPPCT30	165/169/171/175	221	191/193	151/191	191/193
BPPCT032	173/175/181/183	190	200	163	194/200/202
UDP96-005	134/138/154/156/158/164/175	130/132	158/164/175	122	156/173
UDP96-013	149/151	212	179	179/224/226/261	163/179
pchgms2	145/151/155/161/171	181/185	155	126	155/157
CPPCT33	142/144/146/148	161	152	137	142/148/150/152
UDP97-401	100/106/110	98	121/123	121/123/125/172	119/121
CPPCT16	175/195/197/199	179	193	183	185/193/205
BPPCT001	132	130	154/160	146/152	152/156/162
pchgms1	194/208	200	191	183/191	191/193
pchgms4	141/155/173	165/169	173	150/165/173	173/175
UDP96-001	108/110/139	106	125	86/96/104	117/119/125
UDP98-412	94/98/106/109/115/117/121	113	125	86/92	113/119/125/127
BPPCT034	205/213/228/236/240	207	226/238	224/238/246	224/238
pchgms3	182/186/194	169/175	204/206	198/200/204/206	175/182/190/204
BPPCT025	147/153/171/179/185	183/185	175	175/177/181/191/193	171/187/189/191/193
BPPCT023	179/211/215/225/227/229	215	181	181/215	181/211

Fig. 2. Dendrogram of 29 accessions in *Prunus* resulting from the unweighted pair-group method with arithmetic means (UPGMA) cluster analysis based on the similarity coefficient obtained from simple sequence repeat (SSR) data. Twenty-nine accessions (codes 1–29) are divided into five clusters corresponding to five species (*P. davidiana*, *P. kansuensis*, *P. ferganensis*, *P. mira*, and *P. persica*).

DEVELOPMENT OF A PEACH-RELATED SPECIES GENOTYPE DATABASE USING FLUORESCENT-LABELED SSR MARKERS. The 18 SSR markers were used in fluorescent PCR and CE analysis, and a genotype database was generated for 20 representative accessions based on the 18 SSR markers (Table 4), then the genotype database for five species was generated based on the 18 SSR markers and 20 representative accessions (Table 5). The discrimination ability of 18 loci in the database varied and ranked from high (BPPCT010) to low (BPPCT023). The first five markers had high discrimination power and could be useful for the generation of a standard profile for species identification. Marker BPPCT010 can discriminate five species, and any marker of CPPCT30, BPPCT032, UDP96-005, and UDP96-013 can be used to discriminate two species (Table 5). Adoption of the other markers for species identification would facilitate

data sharing and help to correct for variation in data analysis between laboratories. The species *P. kansuensis* was the most easily identified among the five related species, and was one of the species that could be discriminated with most of the 18 selected SSR loci. The species *P. mira*, *P. davidiana*, *P. ferganensis*, and *P. persica* can also be discriminated by their unique loci (Table 5).

IDENTIFICATION OF UNKNOWN SAMPLE SPECIES. All the allele sizes of the eight unknown peach samples (codes 21–28) and 21 known accessions (codes 1–20, code 29) are presented in Table 4. A dendrogram of 29 samples was established (Fig. 2). Eight anonymous samples were included in three species clusters: samples 21, 22, and 23 were in the *P. davidiana* cluster; samples 24, 25, and 26 were in the *P. ferganensis* cluster; and samples 27 and 28 were in the *P. persica* cluster. As expected, sample 29, which was a *P. persica* cultivar, was found in the *P. persica* cluster. It was therefore possible to identify species by analysis with 18 SSR loci and phylogenetic comparison with the 20 known representative samples.

INTERSPECIFIC HYBRID ANALYSIS. Early identification of hybrids is desirable in peach cross breeding, and the selected 18 SSR markers can be used for hybrids analysis at the seedling stage. The F_1 hybrids share one allele with each of their parents on each locus, and progeny genotype(s) can therefore be predicted and proved by their parental genotypes. For example, only one genotype occurs in the F_1 hybrid on the locus of BPPCT010 in the hybridization of *P. persica* $\times$ *P. ferganensis* (Fig. 3A), two genotypes on the locus of UDP96-005 in the hybridization of *P. persica* $\times$ *P. kansuensis* (Fig. 3B), and four genotypes on the locus of UDP96-005 in the hybridization of *P. persica* $\times$ *P. davidiana* (Fig. 3C).

Discussion

Peach cultivars can be easily distinguished using a unique DNA fingerprint (Li et al., 2013) or by molecular identity (Chen et al., 2011), because commercial peach clones are propagated by grafting. Peach-related species are wild resources in a mixed

Fig. 3. ABI genotyper (Applied Biosystems, Foster City, CA) output of simple sequence repeat (SSR) profile of parents and progeny using for interspecific hybrid genotype analysis in *Prunus*. Allele sizes of the parents and their F₁ hybrid(s) are indicated at each SSR loci: (A) profile for BPPCT010 of *P. persica*, *P. ferganensis*, and F₁ hybrid (*P. persica* × *P. ferganensis*); (B) profile for UDP96-005 of *P. persica*, *P. kansuensis*, and F₁-hybrids (*P. persica* × *P. kansuensis*); (C) profile for UDP96-005 of *P. persica*, *P. davidiana*, and F₁-hybrids (*P. persica* × *P. davidiana*). The x- and y-axes show allele size (in bps) and fluorescence intensity, respectively.

population that are commonly propagated by seed. We fully considered the sample origin, amount, and representativeness in this study. The 20 accessions used to screen for optimal SSRs all came from NGRPC, and different type and origin accessions were used in *P. davidiana* (red flower and white flower), *P. kansuensis* (red root and white root), *P. mira* (in Tibet and in Sichuan) and *P. persica* (in Hubei, Guizhou, Sichuan, and Hebei). Four accessions is a small sample; however, they were the recommended representative accessions from the three NGRPCs and should be considered as a starting point to which data from additional accessions and alleles can be added. Eight unknown accessions were tested for species identification, and three known interspecific hybrid populations were tested for hybrid analysis in this study.

Nine to ten representative loci were initially proposed in each *Prunus* linkage group; thus, a total of 78 SSRs were used as candidates. No more than 20 SSRs for validation were proposed prescreening with 20 representative accessions, resulting in 18 SSRs being finally selected on the basis of their amplification reliability and discrimination power. Some highly polymorphic SSRs from previous publications, such as BPPCT020 (Chen et al., 2011) and CPPCT22 (Bouhadida et al., 2011), were excluded from the current SSR panel. Use of loci with relatively few alleles simplified germplasm identification and increased efficiency of identification. Three known interspecific hybrids were used to evaluate transferability between species of the SSRs panel, and the results were in accord with their real identities.

A basic genotype database was established based on the 18 selected SSRs and 20 representative accessions, which should be useful for the identification of unknown *Prunus* accessions using dendrogram construction and cluster analysis. Fluorescent labeling and CE analyses improved the efficiency and accuracy of species identification in the current study. As allele size

validation can be affected by primer tailing fluorescence chemicals, multiplexing PCR, and PCR product analysis (agarose gel, polyacrylamide gel, or capillary systems), SSR screening streamlining and database development for unknown samples identification and hybrid analysis using the same FAM-labeled forward primers, single-multiplexing PCR, and automatic CE analysis were used in this study. In fact, the relative range of allele sizes for five species on each locus is independent on the method used, which can be used as reference for species identification (Table 5). Eight unknown samples were successfully identified by the ZFRI, CAAS, which demonstrated the reliability of the genotype database and the SSRs panel.

Some peach-related species have disappeared from their original habitats in recent years due to climate change, over-exploitation, pollution, and agricultural land reclamation (Xing et al., 2015; Zhong, 2008). On the other hand, accessions of species related to peach are preserved less frequently than those of cultivated peach, which are preserved at NGRPC. The practical genotyping developed in this study for five peach-related species will facilitate investigation and preservation of further wild accessions and allow the breeding potential of peach-related species to be fully realized. Peach-related species identification will be further enhanced by the availability of the peach genome sequence (Verde et al., 2013), the express sequence tag SSR database (Chen et al., 2014), microsatellite maps for the whole peach genome, and functional mining of genes associated with SSRs (Dettori et al., 2015; Zalapa et al., 2012).

Conclusions

In this study, 20 representative accessions at NGRPC were used to select for 18 SSRs, which were then successfully used for the efficient genotyping of five peach-related species

including *P. mira*, *P. davidiana*, *P. kansuensis*, *P. ferganensis*, and *P. persica*. Based on the 18 SSRs and 20 representative accessions, a genotype database of the five species was established using fluorescent labeling and CE analysis. The SSR panel and genotype database were successfully used for the identification of eight unknown peach samples and three known interspecific hybrids analysis. The work presented provides a useful reference profile that should help facilitate peach germplasm management and peach breeding.

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