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Genetic diversity analysis of sweet kernel apricot in China based on SSR and ISSR markers

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ABSTRACT. Simple sequence repeat (SSR) and inter-simple sequence repeat (ISSR) markers were used to evaluate genetic diversity among 22 sweet kernel apricot accessions and 12 cultivars in China to provide information on how to improve the utilization of kernel apricot germplasms. The results showed that 10 pairs of SSR primers screened from 40 primer pairs amplified 43 allelic variants, all of which were polymorphic (100%), and 9 ISSR primers selected from 100 primers amplified 67 allelic variants with 50 polymorphic bands (74.63%). There was a relatively distant genetic relationship between the 34 samples, where their genetic similarity coefficient was between 0.62 and 0.99. The UPGMA dendrogram constructed using combined data of the two marker systems separated the genotypes into three main clusters.

Key words: Kernel apricot; Simple sequence repeat; Genetic diversity; Inter-simple sequence repeat

INTRODUCTION

Kernel apricot is one the six woody grain and oil strategic species. Among them, sweet kernel apricot is our unique economic species, which originated in Zhuolu, Hebei Province, China. The existing germplasm is less at present but has important economic value and prospects for wide utilization because of its thin endocarp, sweet kernels and sources of high-quality oil. Its kernel oil is a healthy cooking oil. The unsaturated fatty acid content of the oil is about 95%, and the monounsaturated fatty acid content of it is more than 70%. At the same time, it is rich in proteins, vitamins, minerals, dietary fiber and trace elements required for the human body.

Genetic diversity analysis is essential for breeding, conservation and selection of appropriate genotypes of this important species. Molecular markers have been proven to be powerful tools. A variety of PCR-based DNA markers, such as random-amplified polymorphic DNA, microsatellite or simple sequence repeat (SSR), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism, and sequence-related amplified polymorphism, provide the opportunity for fine-scale genetic characterizations, and still generate a large amount of data in a short period of time. Therefore, they are frequently used for genetic diversity assessment (Maguire et al., 2002; Gillaspie et al., 2005), variety identification (Hurtado et al., 1999; Bassil and Postman, 2010), genetic map construction (Piquemal et al., 2005; Celyon et al., 2008), pedigree analysis (Dreisigacker et al., 2004), etc.

SSR markers, also called microsatellite DNA markers or short tandem repeat markers are hypervariable DNA elements, which consist of tandemly repeated mono-, di-, tri-, tetra-, or pentanucleotide motifs. They are abundantly present in the genomes of higher organisms (Tautz et al., 1989). SSR markers have a number of advantages, such as the high level of polymorphisms, locus specificity, co-dominance, reproducibility, convenience through using PCR, and random distribution throughout the genome (Powell et al., 1997). As such, they have become a popular marker system in rice, wheat, almond, peach, and apple (Jain et al., 2004; Khlestkina et al., 2004; Xie et al., 2010; Garkava-Gustavsson et al., 2013). ISSR markers use primers based on di-, tetra-, or pentanucleotide sequence repeats and anchored nucleotides. These are randomly selected with the advantage of analyzing multiple loci in a single PCR and not requiring any prior information on the target sequence in the genome. They have been widely used in recent years in wheat, bean, firs, and tea (Hailu et al., 2005; Marotti et al., 2007; Zhang et al., 2007; Liu et al., 2010).

However, no information has been published concerning the genetic diversity of kernel apricot. The objectives of this study were to employ SSR and ISSR markers for investigation of genetic relationships and diversity between 22 sweet kernel apricot accessions and 12 cultivars in China. Comparisons were also made between the two molecular markers to assess their efficacy in determining the levels of genetic diversity.

MATERIAL AND METHODS

DNA extraction

Leaf samples of 22 sweet kernel apricot accessions and 12 cultivars were collected from Zhuolu (40°37'N, 115°2'E), Hebei Province and Luoyang (34°39'N, 112°24'E), Henan Province, China (Table 1). Genomic DNA was extracted from leaf tissue by the cetyltrimeth-

ylammonium bromide (CTAB) method as described by Saghai Maroof et al. (1984). The concentration of extracted DNA was estimated using 0.8% agarose gel electrophoresis. The samples were adjusted to 50 ng/μL and stored at -20°C prior to PCR amplification.

Table 1. Origins of 22 sweet kernel apricot accessions and 12 cultivars in this study.

Identification	Origin	Identification	Origin
Yiwofeng	HBZL	HB-18	HBZL
Chaoren	HBZL	HB-19	HBZL
Changchengyihao	HBZL	HB-20	HBZL
80A03	HBZL	HB-21	HBZL
Youyi	HBZL	HB-22	HBZL
Weixuanyihao	HBZL	HN-23	HNLY
Sanganqi	HBZL	HN-24	HNLY
Longwangmao	HBZL	HN-25	HNLY
Fengren	HBZL	HN-26	HNLY
Bokeyihao	HBZL	HN-27	HNLY
Baiyubian	HBZL	HN-28	HNLY
79C13	HBZL	HN-29	HNLY
HB-2	HBZL	HN-30	HNLY
HB-3	HBZL	HN-32	HNLY
HB-8	HBZL	HN-33	HNLY
HB-9	HBZL	HN-34	HNLY
HB-15	HBZL	HN-35	HNLY

HBZL= Zhuolu, Hebei Province; HNLY = Luoyang, Henan Province.

Primer selection

Initially, 40 pairs of SSR primers from Rosaceae universal primers and 100 ISSR primers [University of British Columbia (UBC), Vancouver, Canada (set #9)] were tested with 12 samples for PCR amplification. On the basis of the maximum number and stability of polymorphic bands, 10 pairs of SSR primers and 9 ISSR primers were selected for further characterization of 34 genotypes.

SSR and ISSR analysis

SSR-PCR was performed in a 20-μL reaction mixture containing 2.0 μL 10X PCR buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 1.5 U Taq DNA polymerase, 0.5 μM of each primer, and 50 ng genomic DNA. The cycling parameters were programmed to 1 cycle of 94°C for 5 min, 35 cycles of 94°C for 45 s, optimal annealing temperature for 45 s, and 72°C for 90 s, followed by 72°C for 10 min.

The products were separated by 8% polyacrylamide gel electrophoresis with 1X TBE buffer using Fluorescent Imaging Technology (Liu et al., 2011). The first procedure was that PCR products were mixed with 2 μL 6X loading buffer containing Gelred dye in a PCR tube. It was then sampled and electrophoresed for 3 h at 120 V constant voltage. After washing the double-sided glasses, the gels were peeled, rinsed and placed in an ultraviolet analysis device (background, with glass board) for photographic analysis.

ISSR-PCR was performed in a 25-μL reaction mixture containing 2.5 μL 10X PCR buffer, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 2.0 U Taq DNA polymerase, 0.5 μM of each primer, and 60 ng genomic DNA. The cycling parameters were programmed to 1 cycle of

94°C for 5 min, 40 cycles of 94°C for 60 s, optimal annealing temperature for 60 s, and 72°C for 120 s, followed by 72°C for 10 min. Amplified products (5 µL) were fractionated on 1.5% agarose gels, stained with Gelred and photographed under UV light (Wang et al., 2009).

Data analysis

ISSR and SSR bands were scored as present (1) or absent (0). Only the clearest and strongest reproducible bands were scored and used for the analysis. Among them, different ISSR phenotypes were put into the POPGENE program version 1.32 (Yeh et al., 1997). The following indices were used to quantify the amount of genetic variability between the 34 genotypes: percentage of polymorphic bands (PPB), observed number of alleles (N_A), effective number of alleles (N_E), Nei's gene diversity (h), and Shannon information index (I).

The NTSYS-pc software ver. 2.11 (Rohlf, 2004) was used to calculate Nei's similarity coefficients between all the samples based on the combined data of SSR and ISSR markers. Similarity matrices were used to construct the unweighted pair group method with arithmetic average (UPGMA) dendrograms within the SAHN module of the NTSYS program.

RESULTS

Levels of polymorphism and discriminating capacity of the assays

The results of two molecular assays in the fingerprinting of the 34 samples are presented in Tables 2 and 3. In the SSR method, a total of 43 bands were amplified by the 10 primers of which all were polymorphic (100%). In this assay, the total number of bands was between 4 and 5 with an average of 4.3. Among the SSR loci, the primers Aprigms18, Pchgms4, and Bppct025 generated the higher number of bands (5 bands), while the other primers had the lower ones (4 bands) (Figure 1).

Table 2. ISSR markers used for the analysis in the study.

Primer	Primer sequence (5'→3')	Total bands	PB	PPB	Annealing T (°C)
Aprigms18	F: TCTGAGTTCAGTGGGTAGCA R: ACAGAATGTGCGTTGCTTTA	5	5	100	55
UDP98-405	F: ACGTCATGAACTGACACCCA R: GAGTCTTTGCTCTGCCATCC	4	4	100	58
UDP98-406	F: TCGGAAACTGGTAGTATGAACAGA R: ATGGGTCGTATGCACAGTCA	4	4	100	58
UDP98-409	F: GCTGATGGGTTTTATGGTTTTC R: CGGACTCTTATCCTCTATCAACA	4	4	100	57
UDP98-411	F: AAGCCATCCACTCAGCACTC R: CCAAAAACCAAAACCAAAGG	4	4	100	56
Pchgms4	F: ATCTTCACAACCCTAATGTC R: GTTGAGGCAAAAGACTTCAAT	5	5	100	54
Pchgms5	F: CGCCCATGACAAACTTA R: GTCAAGAGGTACACCAG	4	4	100	54
Bppct007	F: TCATTGCTCGTCATCAGC R: CAGATTTCTGAAGTTAGCGGTA	4	4	100	56
Bppct025	F: TCCTGCGTAGAAGAAGGTAGC R: CGACATAAAGTCCAAATGGC	5	5	100	58
Bppct030	F: AATTGTACTTGCCAATGCTATGA R: CTGCCTTCTGCTCACACC	4	4	100	57
Average		4.3	4.3	100	

PB = polymorphic bands; PPB = percentage of polymorphic bands.

Table 3. ISSR markers used for the analysis in the study.

Primer	Primer sequence (5'→3')	Total bands	PB	PPB	Annealing T (°C)	N_A	N_E	h	*I*
UBC825	ACACACACACACACACT	8	6	75.00	52	1.7500	1.4447	0.2624	0.3951
UBC834	AGAGAGAGAGAGAGAGYT	7	6	85.71	55	1.7969	1.4347	0.2548	0.3907
UBC840	GAGAGAGAGAGAGAGAYT	6	5	83.33	52	1.8333	1.4691	0.2861	0.4342
UBC841	GAGAGAGAGAGAGAGAYC	9	9	100.00	54	2.0000	1.6036	0.3503	0.5200
UBC842	GAGAGAGAGAGAGAGAYG	7	4	57.14	52	1.5714	1.3238	0.1850	0.2731
UBC847	CACACACACACACACARC	7	5	71.43	52	1.7143	1.3771	0.2314	0.3550
UBC850	GTGTGTGTGTGTGTGTYC	8	5	62.50	47	1.6250	1.3625	0.2177	0.3284
UBC856	ACACACACACACACACYA	8	5	62.50	42	1.6250	1.3565	0.2208	0.3346
UBC860	TGTGTGTGTGTGTGTGRA	7	5	71.43	52	1.7143	1.3127	0.2027	0.3170
Average		7.4	5.5	74.34		1.7367	1.4094	0.2757	0.3720

PB = polymorphic bands; PPB = percentage of polymorphic bands; N_A = number of alleles; N_E = effective number of alleles; H = Nei's gene diversity; I = Shannon information index.

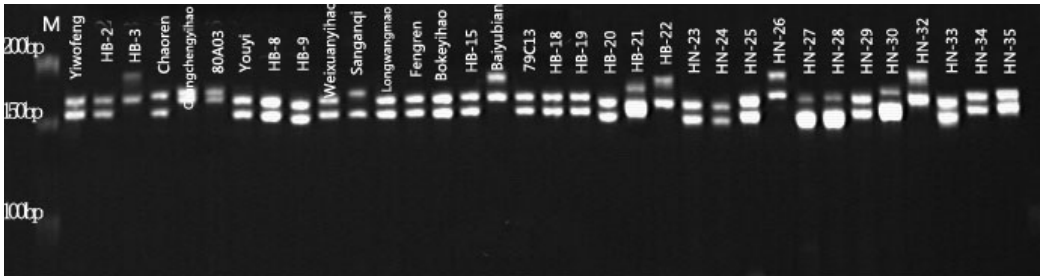

Figure 1. Amplification products for 34 samples by the SSR primer Bppct030. *Lane M* = 1-kb DNA ladder.

In ISSR analysis, a lower level (74.34%) of polymorphism was obtained, compared to the SSR method. Among them, the primer UBC841 detected the highest polymorphism (100%) and the primer UBC842 detected the lowest polymorphism (57.14%). A total of 67 bands were revealed by 9 ISSR primers based on clarity and specificity, with an average of 7.4 bands per locus. Among the ISSR loci, the highest polymorphism was observed with primer UBC841 (9 bands), while the lowest one was with primer UBC840 (6 bands). The average N_A and N_E were 1.7367 and 1.4094, respectively. *h* for individual loci varied from 0.1850 (UBC842) to 0.3503 (UBC841) with an average of 0.2757, and the *I* value was from 0.2731 (UBC842) to 0.5200 (UBC841) with an average of 0.3720 (Figure 2).

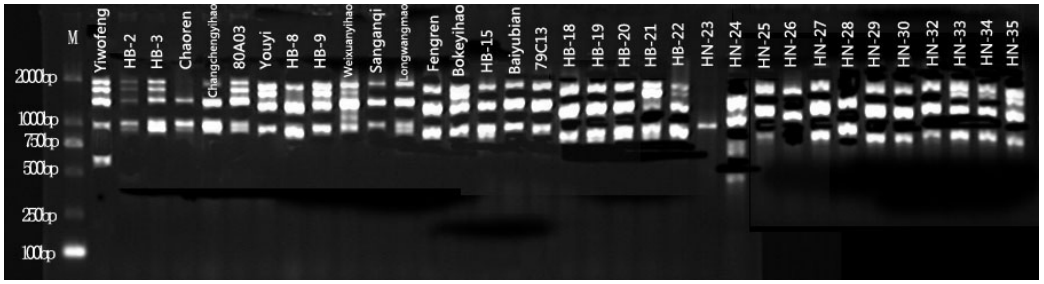

Figure 2. Amplification products for 34 samples by the ISSR primer UBC860. *Lane M* = 2-kb DNA ladder.

Clustering and genetic relationships among 34 genotypes

A total of 110 polymorphic bands were analyzed for genetic similarity coefficient (GSC) between the 22 sweet kernel apricot accessions and 12 cultivars in China. The results showed that genetic relationships were relatively distant between the 34 samples in this study. GSC ranged from 0.62 to 0.99 on the basis of the combined data of the SSR and ISSR molecular markers. The closest genetic relationship was between HB-18 and HB-20 with GSC of 0.99, and the farthest genetic relationship was between HB-3 and HN-25 with GSC of 0.62. GSC of most varieties was from 0.7 to 0.8. The 34 samples were clearly classified into three distinct major groups through cluster analysis based on GSC: Changchengyihao and HB-3 each as one cluster, and the other samples as the third cluster (Figure 3).

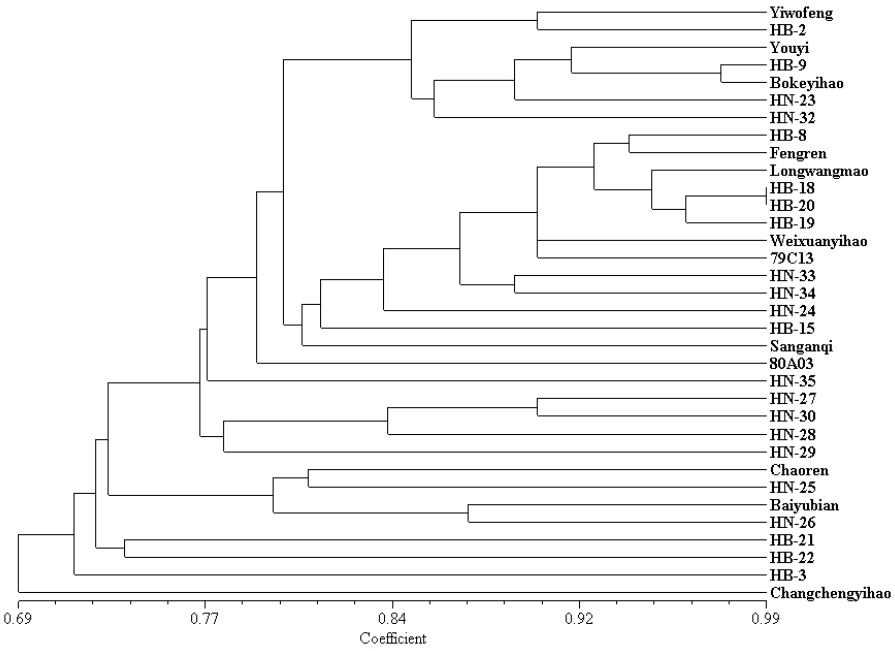

Figure 3. UPGMA cluster for 34 varieties based on combined data of SSR+ISSR markers.

DISCUSSION

Considerable research has been on the genetic relationship of *Armeniaca* Scop. in recent years, but the genetic relationship of the sweet kernel apricot germplasm resources was the first study. In SSR analysis, a higher level (100%) of polymorphism than with ISSR method was detected (74.34%), which could be attributed to the significantly lower number of SSR alleles analyzed. The values of microsatellite variation in the sweet kernel apricot germplasm resources were greatly higher than in other species of *Armeniaca* Scop. in China using SSR molecular markers. The polymorphism level of SSR used in this study was higher than in the study of He et al. (2006) (PPB = 84.9%) and Liu et al. (2013) (PPB = 93.0%). However,

the polymorphism level of ISSR was lower than that reported by Feng et al. (2005) (PPB = 90%) and Xie et al. (2011) (PPB = 96.5%). In ISSR analysis, *h* with an average of 0.2757 and *I* with an average of 0.3720 showed that there was abundant genetic diversity between the sweet kernel apricot germplasm resources.

The UPGMA dendrogram showed a relatively far relationship between 22 sweet kernel apricot accessions and 12 cultivars based on the GSC range from 0.62 to 0.99. There was no clear separation of the varieties according to their collection location, perhaps because the resources of Luoyang, Henan Province, were introduced from Zhuolu Hebei Province. The 34 samples were classified into three distinct major groups on the basis of GSC: Changchengyihao and HB-3 each one cluster, and the other samples as the third cluster. The results showed that genetic relationship was distant between HB-3 and Changchengyihao and the other species, maybe because HB-3 and Changchengyihao were early in evolution. We should pay more attention to them and use them in breeding new varieties. Meanwhile, there was a close genetic relationship between HB-18 and HB-20 with GSCs 0.99, which showed that they were maybe the same variety. We should check them according to morphological characters.

As an important economic forest tree species, loss of genetic diversity could not support enough breeding resources for the development of sweet kernel apricot germplasm resources. Meanwhile, the information on the levels and distribution of genetic diversity was important for designing conservation strategies for this species. Therefore, it was necessary to study the genetic relationship and genetic diversity for its genetics and breeding research.

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