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Genetic Diversity and Population Structure of Chinese Jujube (*Ziziphus jujuba* Mill.) and Sour Jujube (*Ziziphus acidojujuba* Mill.) using Inter-simple Sequence Repeat (ISSR) Markers

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Abstract The Chinese jujube (*Ziziphus jujuba* Mill.) originates from sour jujube (*Ziziphus acidojujuba* Mill.) and is an economically important genus in the Rhamnaceae family. However, little is known about the genetic relationship between jujube cultivars and wild species. In this study, we estimated the genetic variation and relationships between 85 jujube cultivars and 55 sour jujube individuals by ISSR markers. Of 216 ISSR primers, 110 were able produce amplified product(s) and 28 showed polymorphisms, accounting for 50.9% and 25.5% of total primers respectively. A total of 89 amplicons were amplified with 28 primers, of which 42 amplicons (47.2%) were polymorphic, and most of primers exhibited high PIC values. Cluster analysis and population structure analysis roughly divided the 140 accessions into two major groups. One group included all jujube cultivars and some sour jujube individuals, and the other group included remaining sour jujube individuals. Most jujube cultivars have a weak correlation with their origin, and there are obvious gene exchanges between sour jujube and jujube cultivars. The results provide a useful basis for jujube germplasm conservation, genetic improvement and evolution research.
Keywords *Ziziphus jujuba* Mill; *Ziziphus acidojujuba* Mill; ISSR; Genetic diversity; Population structure

Background

Chinese jujube (*Ziziphus jujuba* Mill.) and sour jujube (*Ziziphus acidojujuba* Mill.) belong to the family Rhamnaceae. Chinese jujube (hereafter referred to as jujube) is an economically and ecologically important species that is a popular fruit tree in Asia (Qu and Wang, 1993). According to archaeological evidence, jujube, which has been cultivated for more than 3,000 years, originated in China (Qu and Wang, 1993; Liu, 2003; Liu and Wang, 2009; Li et al., 2013). As one of the oldest cultivated fruit trees, the germplasm resources of jujube are abundant, with more than 900 cultivars reported thus far (Liu and Wang, 2009). Jujube fruits have high nutritional value and a long history of usage as an edible fruit and in herbal medicine, and constitute a rich source of vitamin C, cAMP, flavonoids, triterpenic acids, and polysaccharides (Gao et al., 2013). Recent phytochemical and pharmacological studies have revealed that the main biologically active components of jujube fruits are beneficial to the human health (Choi et al., 2012; Chen et al., 2017a). Sour jujube, also known as wild jujube, is another important species that is regarded as the wild ancestor of jujube (Qu and Wang, 1993; Liu, 2003; Liu and Wang, 2009). It is widely planted as the rootstock for jujube and its seeds have high medicinal value (Qu and Wang, 1993; Islam and Simmons, 2006; Liu and Wang, 2009; Zhang et al., 2015a). Research on the genetic diversity and phylogenetic relationships of jujube is beneficial for jujube breeding and will help to elucidate the evolutionary history of jujube.

With the development of molecular biology and technology, the genetic diversity and genetic structure of jujube have been studied using molecular markers, including amplified fragment length polymorphism (AFLP), chloroplast microsatellite (cpSSR), random amplified polymorphic DNA (RAPD), sequence-related amplified polymorphisms(SRAPs), simple sequence repeat (SSR), single nucleotide polymorphism (SNP), and so on (Peng et al., 2000; Bai, 2008; Ma et al., 2011; Soliman et al., 2013; Li et al., 2014; Wang et al., 2014; Xie, 2014; Huang et al., 2015; Xiao et al., 2015; Zhang et al., 2015c; Fu et al., 2016; Xu et al., 2016; Chen et al., 2017b). For

example, 30 main cultivars were divided into six groups based on AFLP analysis (Xie, 2014). The genetic diversity of 76 jujube cultivars was analyzed using 31 SSR markers, and the cultivars were divided into three main groups based on cluster analysis (Wang et al., 2014). One hundred and forty accessions were clustered into two groups by STRUCTURE Software 2.3.4 (http://web.stanford.edu/group/pritchardlab/structure.html) and principal coordinate analyses (PCoA, https://www.xlstat.com/en/solutions/features/principal-coordinate-analysis) based on SNPs (Chen et al., 2017b). However, only a few studies involving the genetic diversity and genetic structure of sour jujube and the genetic relationship between jujube and sour jujube have been reported (Huang et al., 2015; Zhang et al., 2015a).

The inter simple sequence repeat (ISSR) technique is a polymerase chain reaction (PCR)-based method that involves the amplification of regions between adjacent, inversely oriented microsatellites using single sequence repeats, usually 16-25 bp long, as primers (Zietkiewicz et al., 1994). It is a rapid, simple, and inexpensive way to study genetic diversity, phylogeny, and evolutionary biology (Reddy et al., 2002). The jujube genome contains high-density simple sequence repeats (Liu et al., 2014); therefore, it is suitable for genetic diversity analysis using ISSR markers. In the present study, the genetic diversity and population structure of 85 jujube cultivars and 55 sour jujube individuals were analyzed by ISSR markers. The results revealed the level of genetic diversity in the collections and the genetic relationships between jujube and sour jujube.

1 Results

1.1 Detection of polymorphisms

All 216 of the ISSR primers were evaluated for successful PCR amplification by testing three accessions. Among them, 110 primers (50.9%) successfully amplified at least one clear and stable fragment from the jujube and sour jujube genome. To test the polymorphism of the 110 ISSR primers, 12 jujube cultivars and 12 sour jujube individuals were further analyzed. Of the 110 ISSR primers, 28 primers (25.5%) were polymorphic (Figure 1) and produced a total of 89 DNA fragments (Table 1). The number of amplified fragments varied from 2 to 6 with an average of 3.19 amplicons per primer, and their sizes ranged between 200 and 1,500 bp (Table 1). The polymorphism per primer ranged from 16.7 (ISSR60) to 100% (ISSR-11 and ISSR-13) and the average number of polymorphic bands per primer was 1.5 (Table 1). Based on genetic variation standards (Botstein et al., 1980), the polymorphism information content (PIC) values calculated ranged from 0.168 to 0.777, and most of the primers exhibited high PIC values (Table 1). Thus, our results indicated that ISSR markers could be used to assess the genetic diversity and population structure in these germplasms.

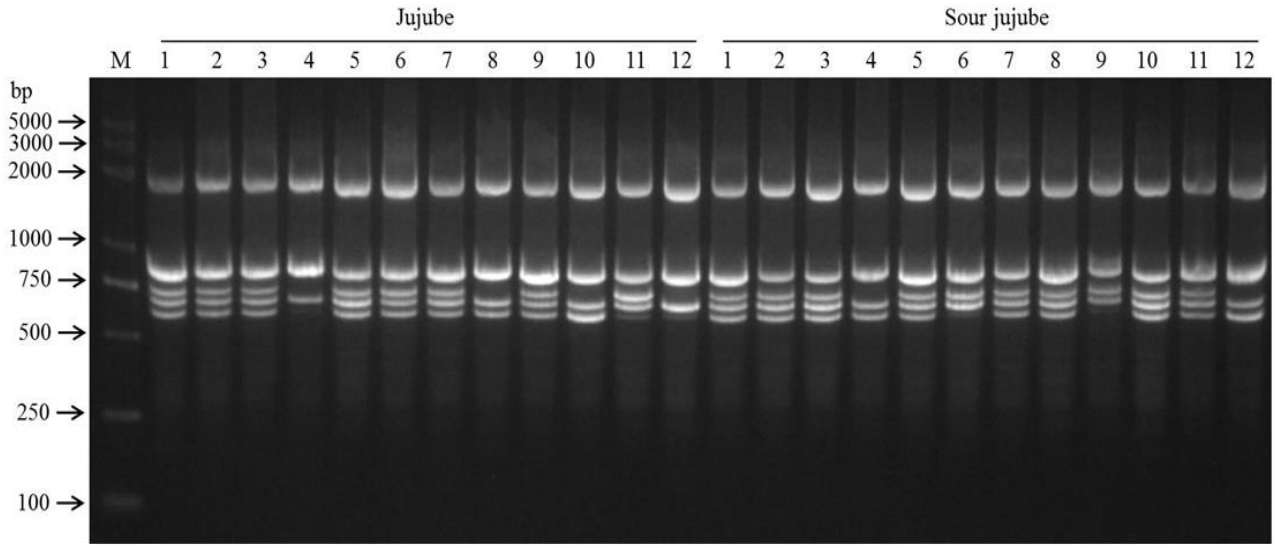

Figure 1 Amplification products from 12 jujube cultivars and 12 sour jujube individuals using the ISSR-25 primer. M: D2000 plus DNA Ladder (Solarbio, Beijing, China)

Table 1 The 28 ISSR primers selected for this study

Primer name	Primer	Annealing temperature (°C)	Allele range (bp)	Total no. of bands	No. of polymorphic bands	PIC
ISSR11	GAGAGAGAGAGAGAGAC	50	550-600	2	2	0.684
ISSR13	CTCTCTCTCTCTCTCTT	50	700-800	2	2	0.507
ISSR22	TCTCTCTCTCTCTCTCA	50	700-900	2	1	0.396
ISSR23	TCTCTCTCTCTCTCTCC	50	600-1,000	3	1	0.436
ISSR25	ACACACACACACACACT	50	650-950	5	3	0.771
ISSR27	ACACACACACACACACG	50	450-950	3	2	0.722
ISSR40	AGAGAGAGAGAGAGAGTT	55	500-750	2	1	0.382
ISSR43	AGAGAGAGAGAGAGAGTC	55	350-600	5	3	0.777
ISSR46	AGAGAGAGAGAGAGAGTA	55	400-750	3	2	0.693
ISSR47	AGAGAGAGAGAGAGAGGA	55	550-1,500	4	2	0.678
ISSR48	AGAGAGAGAGAGAGAGCA	55	350-1,500	3	1	0.426
ISSR55	GAGAGAGAGAGAGAGATT	55	200-400	2	2	0.639
ISSR57	GAGAGAGAGAGAGAGACT	55	200-350	2	1	0.311
ISSR60	GAGAGAGAGAGAGAGACC	55	200-500	6	1	0.235
ISSR63	GAGAGAGAGAGAGAGACG	55	250-600	4	1	0.414
ISSR66	CTCTCTCTCTCTCTCTAC	55	550-700	2	2	0.64
ISSR68	CTCTCTCTCTCTCTCTAG	55	600-1,500	2	1	0.414
ISSR69	CTCTCTCTCTCTCTCTGG	55	250-500	5	1	0.235
ISSR81	GTGTGTGTGTGTGTGTCC	55	200-1,500	5	1	0.467
ISSR82	GTGTGTGTGTGTGTGTTG	55	300-1,000	3	1	0.275
ISSR88	TCTCTCTCTCTCTCTCGT	55	300-1,000	3	1	0.168
ISSR89	TCTCTCTCTCTCTCTCAG	55	300-700	6	2	0.629
ISSR95	ACACACACACACACACGA	55	600-1,500	2	1	0.402
ISSR103	TGTGTGTGTGTGTGTGGC	55	400-500	2	1	0.488
ISSR105	TGTGTGTGTGTGTGTGGA	55	400-700	2	1	0.496
ISSR121	GATAGATAGACAGACA	50	350-750	3	2	0.733
ISSR124	CTTCACTTCACTTCA	50	400-750	3	2	0.628
ISSR126	GGGTGGGGTGGGGTG	55	550-700	3	1	0.467
Total				89	42	
Average				3.19	1.5	0.504

1.2 Genetic diversity and cluster analysis

To examine the genetic diversity of 140 accessions in detail, we calculated their genetic relationships using Unweighted Pair Group Method and Arithmetic Mean (UPGMA) cluster analysis. Based on the unweighted neighbor-joining clustering, 140 accessions were divided into two major groups (Figure 2).

Group I (G1) contained all of the jujube cultivars and seven sour individuals, and could be further divided into four subgroups. The subgroups under I (G1-I), III (G1-III), and IV (G1-IV) included three jujube cultivars and two sour jujube individuals; two jujube cultivars and one sour jujube individual; and one jujube cultivar and two sour jujube individuals, respectively. Subgroup II (G1-II) included the vast majority of the jujube cultivars and one sour jujube individual, and could be further divided into three clusters. The 41 cultivars in cluster I (C1) mainly originated from northwest China; the 18 cultivars in cluster II (C2) mainly originated from eastern China; and the 14 cultivars in cluster III (C3) mainly originated from central China. Group II (G2) contained the other sour jujube individuals, and could be further divided into four subgroups. These four subgroups (G2-I-IV) included 27, 16, 4, and 1 individual, respectively. The Mantel test showed a weak correlation between genetic divergence and geographical distance (r^2=0.0554, p>0.05). The results showed that the genetic relationships among the different jujube varieties were no significant correlation with the origin of the variety (Figure 2; Table 2).

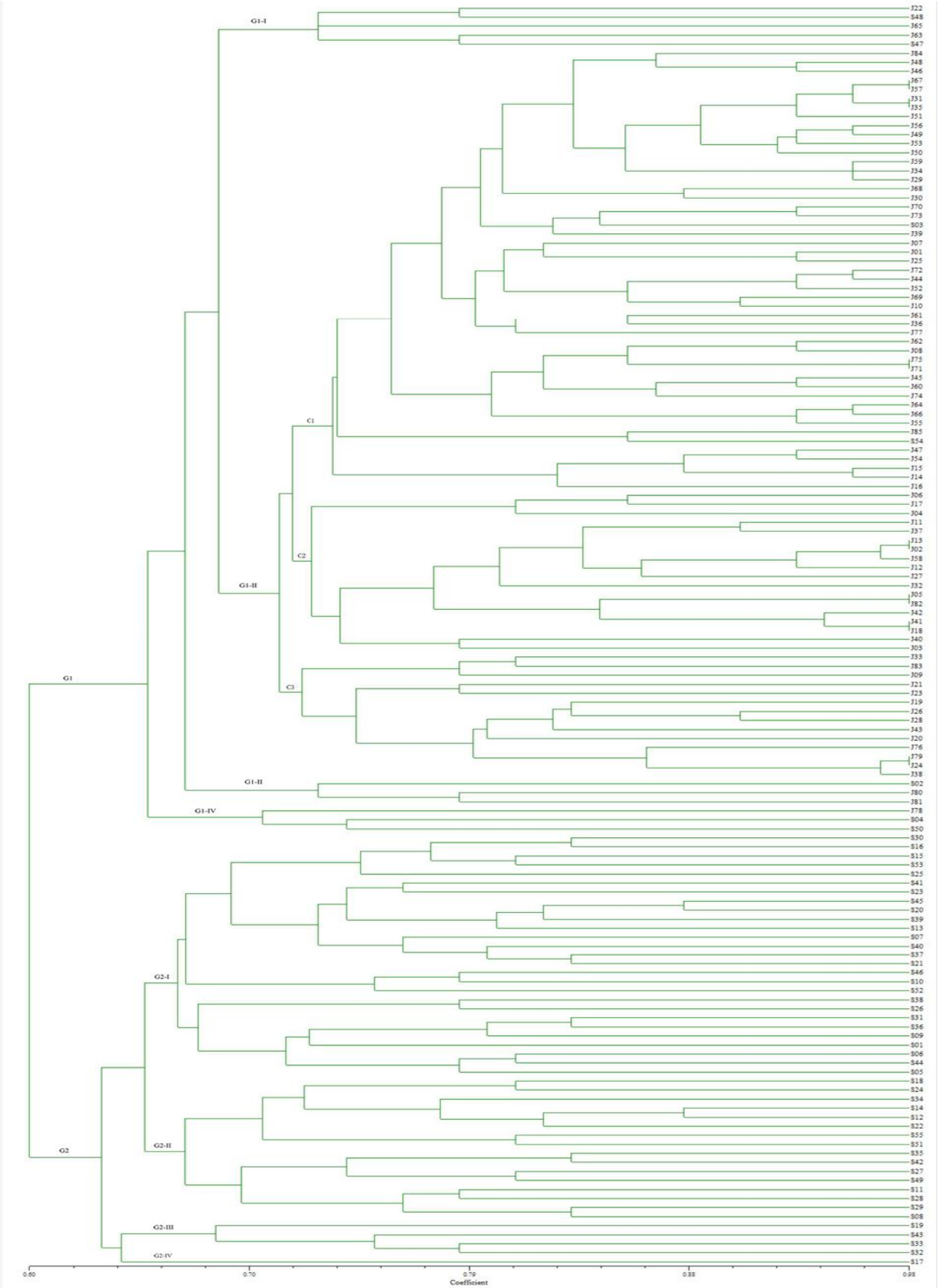

Figure 2 Dendrogram of 140 accessions based on 28 ISSR primers

Table 2 Accessions used in the study

Code	Accession	Species	Usage
J01	Beijingjidanzao	*Ziziphus jujuba* Mill.	Fresh variety
J02	Buluosu	*Ziziphus jujuba* Mill.	Fresh variety
J03	Dabailing	*Ziziphus jujuba* Mill.	Fresh variety
J04	Dabaizao	*Ziziphus jujuba* Mill.	Fresh variety
J05	Daguazao	*Ziziphus jujuba* Mill.	Fresh variety
J06	Dongzao	*Ziziphus jujuba* Mill.	Fresh variety
J07	Fengmiguan	*Ziziphus jujuba* Mill.	Fresh variety
J08	Hunanjidanzao	*Ziziphus jujuba* Mill.	Fresh variety
J09	Jinai NO.3	*Ziziphus jujuba* Mill.	Fresh variety
J10	Lajiaozao	*Ziziphus jujuba* Mill.	Fresh variety
J11	Lengbaiyu	*Ziziphus jujuba* Mill.	Fresh variety
J12	Linqilizao	*Ziziphus jujuba* Mill.	Fresh variety
J13	Pinglujianzao	*Ziziphus jujuba* Mill.	Fresh variety
J14	Qiyuexian	*Ziziphus jujuba* Mill.	Fresh variety
J15	Taigujixinmi	*Ziziphus jujuba* Mill.	Fresh variety
J16	Taianmalingcui	*Ziziphus jujuba* Mill.	Fresh variety
J17	Xiajingmamazao	*Ziziphus jujuba* Mill.	Fresh variety
J18	Xiangfenyuanzao	*Ziziphus jujuba* Mill.	Fresh variety
J19	Xuechengdongzao	*Ziziphus jujuba* Mill.	Fresh variety
J20	Yingluozao	*Ziziphus jujuba* Mill.	Fresh variety
J21	Yongjihamazao	*Ziziphus jujuba* Mill.	Fresh variety
J22	Yuciyazao	*Ziziphus jujuba* Mill.	Fresh variety
J23	Zaoqiangcuizao	*Ziziphus jujuba* Mill.	Fresh variety
J24	Jiaochengtiansuanzao	*Ziziphus jujuba* Mill.	Fresh variety
J25	Chuanlingzao	*Ziziphus jujuba* Mill.	Dry variety
J26	Guantanzao	*Ziziphus jujuba* Mill.	Dry variety
J27	Hongzhaoshiyuehong	*Ziziphus jujuba* Mill.	Dry variety
J28	Jishanliuguanzao	*Ziziphus jujuba* Mill.	Dry variety
J29	Jishanyuanzao	*Ziziphus jujuba* Mill.	Dry variety
J30	Jishanchangzao	*Ziziphus jujuba* Mill.	Dry variety
J31	Jingudazao	*Ziziphus jujuba* Mill.	Dry variety
J32	Jinzandazao	*Ziziphus jujuba* Mill.	Dry variety
J33	Miyunxiaozao	*Ziziphus jujuba* Mill.	Dry variety
J34	Paopaohong	*Ziziphus jujuba* Mill.	Dry variety
J35	Pingshunbenzao	*Ziziphus jujuba* Mill.	Dry variety
J36	Pingyaodazao	*Ziziphus jujuba* Mill.	Dry variety
J37	Pingyaokuduanzao	*Ziziphus jujuba* Mill.	Dry variety
J38	Popozao	*Ziziphus jujuba* Mill.	Dry variety
J39	Pozao	*Ziziphus jujuba* Mill.	Dry variety
J40	Pozaozhibian NO.1	*Ziziphus jujuba* Mill.	Dry variety
J41	Shenglizao	*Ziziphus jujuba* Mill.	Dry variety
J42	Taiguhupingzao	*Ziziphus jujuba* Mill.	Dry variety
J43	Xiaxianziyuanzao	*Ziziphus jujuba* Mill.	Dry variety
J44	Xianxianmuzao	*Ziziphus jujuba* Mill.	Dry variety
J45	Xiangfenmuzao	*Ziziphus jujuba* Mill.	Dry variety
J46	Xiangfenyazao	*Ziziphus jujuba* Mill.	Dry variety

Continuing Table 2

Code	Accession	Origin	Species	Usage
J47	Yuanquzao	Shanxi	*Ziziphus jujuba* Mill.	Dry variety
J48	Yuanlingzao	Shandong	*Ziziphus jujuba* Mill.	Dry variety
J49	Banzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J50	Baodexiaozao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J51	Cangxiantunzizao	Hebei	*Ziziphus jujuba* Mill.	Dual-purpose variety
J52	Cangxianxiaozao	Hebei	*Ziziphus jujuba* Mill.	Dual-purpose variety
J53	Dingxiangxingxingzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J54	Hongzhaoxiaozao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J55	Hupingzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J56	Jiaochengduanzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J57	Jinzao	Shaanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J58	Junzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J59	Lichengdamazao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J60	Lichengxiaozao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J61	Linfentuanzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J62	Linfenzhenhulu	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J63	Ningxiatongxinyuanzao	Ningxia	*Ziziphus jujuba* Mill.	Dual-purpose variety
J64	Qingxuyuanzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J65	Shandongshouzao	Shandong	*Ziziphus jujuba* Mill.	Dual-purpose variety
J66	Taiguduanzizao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J67	Taigudundunzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J68	Taiguheiyezao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J69	Xiaxianyuancuizao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J70	Yucituanzao	Shanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J71	Zanhuangdazao	Hebei	*Ziziphus jujuba* Mill.	Dual-purpose variety
J72	Zanhuangchangzao	Hebei	*Ziziphus jujuba* Mill.	Dual-purpose variety
J73	Zhongyangmuzao	Shaanxi	*Ziziphus jujuba* Mill.	Dual-purpose variety
J74	Ezizao	Zhejiang	*Ziziphus jujuba* Mill.	Sugar treament variety
J75	Jinsizao	Ningxia	*Ziziphus jujuba* Mill.	Sugar treament variety
J76	Nanjingzao	Jiangsu	*Ziziphus jujuba* Mill.	Sugar treament variety
J77	Xuanchengjianzao	Anhui	*Ziziphus jujuba* Mill.	Sugar treament variety
J78	Daguosuanpanzao	Hunan	*Ziziphus jujuba* Mill.	Ornamental variety
J79	Dalilongzao	Shaanxi	*Ziziphus jujuba* Mill.	Ornamental variety
J80	Dashibingzao	Shandong	*Ziziphus jujuba* Mill.	Ornamental variety
J81	Dayewuhezao	Henan	*Ziziphus jujuba* Mill.	Ornamental variety
J82	Longzao	Henan	*Ziziphus jujuba* Mill.	Ornamental variety
J83	Mupanzao	Shaanxi	*Ziziphus jujuba* Mill.	Ornamental variety
J84	Tailihong	Henan	*Ziziphus jujuba* Mill.	Ornamental variety
J85	Taiguhuluzao	Shanxi	*Ziziphus jujuba* Mill.	Ornamental variety
S01	W01	Hebei	*Ziziphus acidojujuba* Mill.	-
S02	W02	Hebei	*Ziziphus acidojujuba* Mill.	-
S03	W03	Hebei	*Ziziphus acidojujuba* Mill.	-
S04	W04	Hebei	*Ziziphus acidojujuba* Mill.	-
S05	W05	Hebei	*Ziziphus acidojujuba* Mill.	-
S06	W06	Hebei	*Ziziphus acidojujuba* Mill.	-
S07	W07	Hebei	*Ziziphus acidojujuba* Mill.	-
S08	W08	Hebei	*Ziziphus acidojujuba* Mill.	-

Continuing Table 2

Code	Accession	Origin	Species	Usage
S09	W09	Hebei	*Ziziphus acidojujuba* Mill.	-
S10	W10	Hebei	*Ziziphus acidojujuba* Mill.	-
S11	W11	Hebei	*Ziziphus acidojujuba* Mill.	-
S12	W12	Hebei	*Ziziphus acidojujuba* Mill.	-
S13	W13	Hebei	*Ziziphus acidojujuba* Mill.	-
S14	W14	Hebei	*Ziziphus acidojujuba* Mill.	-
S15	W15	Hebei	*Ziziphus acidojujuba* Mill.	-
S16	W16	Hebei	*Ziziphus acidojujuba* Mill.	-
S17	W17	Hebei	*Ziziphus acidojujuba* Mill.	-
S18	W18	Hebei	*Ziziphus acidojujuba* Mill.	-
S19	W19	Hebei	*Ziziphus acidojujuba* Mill.	-
S20	W20	Hebei	*Ziziphus acidojujuba* Mill.	-
S21	W21	Hebei	*Ziziphus acidojujuba* Mill.	-
S22	W22	Hebei	*Ziziphus acidojujuba* Mill.	-
S23	W23	Hebei	*Ziziphus acidojujuba* Mill.	-
S24	W24	Hebei	*Ziziphus acidojujuba* Mill.	-
S25	W25	Hebei	*Ziziphus acidojujuba* Mill.	-
S26	W26	Hebei	*Ziziphus acidojujuba* Mill.	-
S27	W27	Hebei	*Ziziphus acidojujuba* Mill.	-
S28	W28	Hebei	*Ziziphus acidojujuba* Mill.	-
S29	W29	Hebei	*Ziziphus acidojujuba* Mill.	-
S30	W30	Henan	*Ziziphus acidojujuba* Mill.	-
S31	W31	Henan	*Ziziphus acidojujuba* Mill.	-
S32	W32	Henan	*Ziziphus acidojujuba* Mill.	-
S33	W33	Henan	*Ziziphus acidojujuba* Mill.	-
S34	W34	Henan	*Ziziphus acidojujuba* Mill.	-
S35	W35	Henan	*Ziziphus acidojujuba* Mill.	-
S36	W36	Henan	*Ziziphus acidojujuba* Mill.	-
S37	W37	Henan	*Ziziphus acidojujuba* Mill.	-
S38	W38	Henan	*Ziziphus acidojujuba* Mill.	-
S39	W39	Henan	*Ziziphus acidojujuba* Mill.	-
S40	W40	Henan	*Ziziphus acidojujuba* Mill.	-
S41	W41	Henan	*Ziziphus acidojujuba* Mill.	-
S42	W42	Henan	*Ziziphus acidojujuba* Mill.	-
S43	W43	Henan	*Ziziphus acidojujuba* Mill.	-
S44	W44	Henan	*Ziziphus acidojujuba* Mill.	-
S45	W45	Henan	*Ziziphus acidojujuba* Mill.	-
S46	W46	Henan	*Ziziphus acidojujuba* Mill.	-
S47	W47	Henan	*Ziziphus acidojujuba* Mill.	-
S48	W48	Henan	*Ziziphus acidojujuba* Mill.	-
S49	W49	Henan	*Ziziphus acidojujuba* Mill.	-
S50	W50	Henan	*Ziziphus acidojujuba* Mill.	-
S51	W51	Henan	*Ziziphus acidojujuba* Mill.	-
S52	W52	Henan	*Ziziphus acidojujuba* Mill.	-
S53	W53	Henan	*Ziziphus acidojujuba* Mill.	-
S54	W54	Henan	*Ziziphus acidojujuba* Mill.	-
S55	W55	Henan	*Ziziphus acidojujuba* Mill.	-

1.3 Population structure

STRUCTURE 2.3.4 (Hubisz et al., 2009) was used to analyze the population structure of jujube and sour jujube accessions. The mean LnP(K) values for the different Ks ranged from 1 to 25, and exhibited a rapid incremental trend before reaching a peak value at K = 2. After K = 2, the mean LnP(K) values gradually increased to K = 25, but variation was observed among the replicate runs. Furthermore, our results showed that the highest value of △K was observed for K = 2, hence all of the accessions could be roughly divided into two major clusters (Figure 3). Using a membership probability threshold of 0.6 (Jakobsson and Rosenberg, 2007; Chen et al., 2012), 94 accessions were assigned to group I, which contained 85 jujube cultivars and 9 sour jujube individuals. The remaining 46 sour jujube individuals were assigned to group II (Figure 4; Table 3).

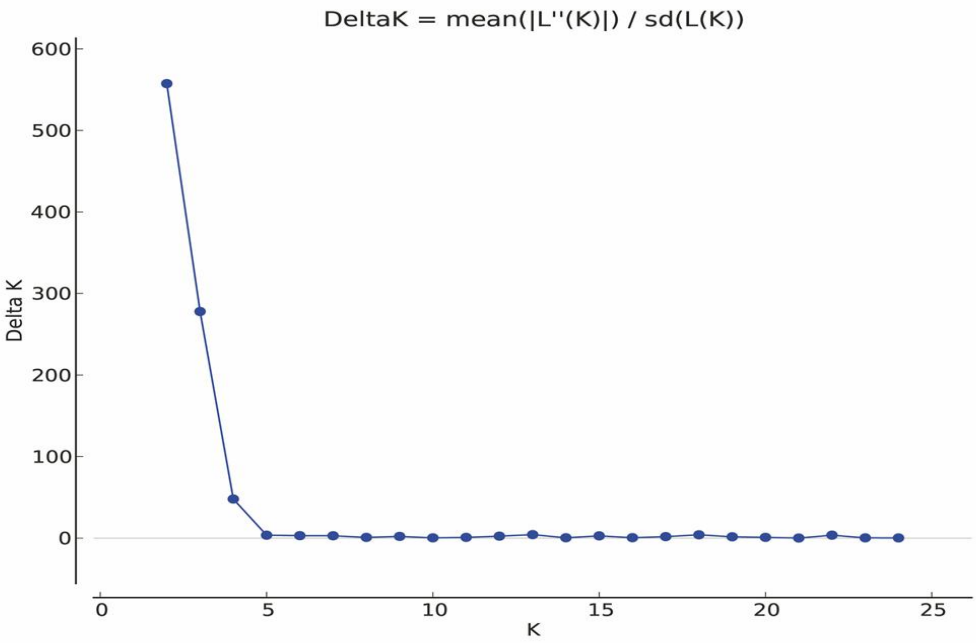

Figure 3 STRUCTURE estimation of the munber of populations for K values ranging from 1 to 25, by delta K (ΔK) values

Figure 4 Population structure (K=2) of 140 accessions

Table 3 Distribution of Q-value of 140 accessions in two groups by model-based cluster method

Group	Number of cultivars in each group	Amount of accessions (%)			
		Q<0.6	Q≥0.6	Q≥0.8	Q≥0.9
I	94	4 (4.3%)	90 (95.7%)	71 (75.5%)	55 (58.5%)
II	46	4 (8.7%)	42 (91.3%)	37 (80.4%)	28 (60.8%)
Total	140	8 (5.7%)	132 (94.3%)	108 (77.1%)	83 (59.3%)

Statistical analysis indicated that the majority of accessions showed strong membership values (Table 4). In group I, 71 accessions (75.5%), including 68 jujube cultivars and three sour jujube individuals, demonstrated shared ancestry. Similarly, 37 individuals (80.4%) had a high proportion of membership in group II. The other accessions showed mixed ancestry from both groups.

Table 4 Summary of genetic variation statistics for 28 ISSR markers from 85 jujube cultivars and 55 sour jujube individuals

Population		N	Na	Ne	I	He	uHe	The percentage of polymorphic loci
Jujube	Mean	85.000	1.810	1.548	0.473	0.317	0.319	88.10%
	SE	0.000	0.085	0.054	0.035	0.026	0.026	
Sour Jujube	Mean	55.000	1.881	1.578	0.492	0.332	0.335	90.48%
	SE	0.000	0.061	0.053	0.033	0.025	0.026	

PCoA also roughly divided the 140 accessions into two clusters (Figure 5), which was consistent with the assignments generated by UPGMA clustering (Figure 2) and population structure analysis (Figure 4). The majority of sour jujube accessions belonging to cluster I were distributed in the left half of the resulting plot. The rest of the sour jujube and all of the jujube accessions belonging to cluster II were distributed in the right of the plot. The distribution of cluster I was more widely scattered than cluster II, indicating that sour jujube had higher diversity than the jujube cultivars.

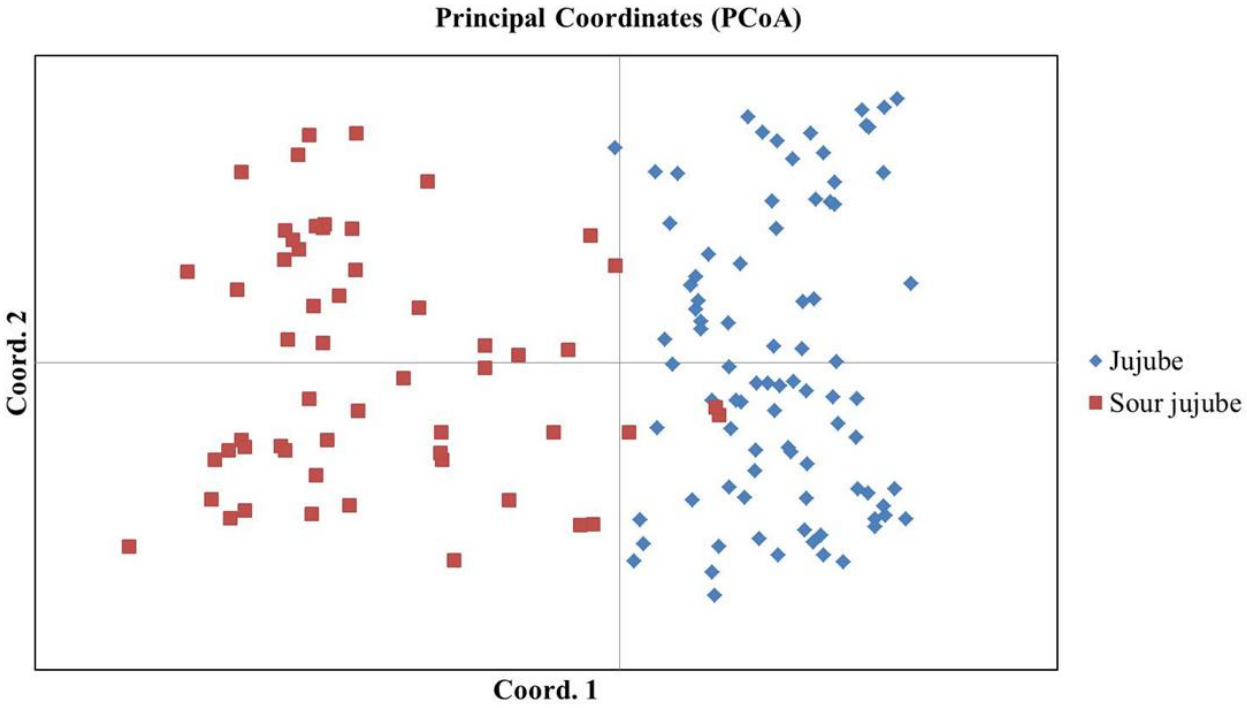

Figure 5 The principal coordinate analysis (PCA) of 140 accessions using ISSR primers

2 Discussion

ISSR marker is a simple and rapid approach that has the advantages of SSR, RAPD, RFLP and AFLP (Reddy et al., 2002). Compared with SSR marker and sequencing technology, it is low cost and does not require prior knowledge of sequence information. ISSR marker has been widely used in the fields of germplasm collection, genetic diversity, genetic mapping and marker assisted selection (Levi et al., 2006; Zhang et al., 2011; Sunar et al., 2016; Cui et al., 2017; Kumar and Roy, 2018). Few studies have focused on elucidating the complex genetic relationships among different jujube varieties by ISSR markers (Singh et al., 2017). In the present study, the genetic diversity of a wide variety of jujube germplasm resources was evaluated, which provides an important scientific basis for the efficient use of these germplasms.

Twenty-eight ISSR markers were used in this study to analyze the genetic diversity of 85 jujube and 55 sour jujube accessions. The results showed that the Shannon's Information Index (I: 0.492) and marker diversity (90.48%) of sour jujube were both higher than in jujube (Table 5). One probable explanation is that the genetic diversity of the jujube varieties has been reduced as a result of long-term evolution and artificial domestication.

Table 5 Distribution of Q-value of 85 jujube cultivars and 55 sour jujube individuals by model-based cluster method

Population	Number of cultivars in each population	Amount of accessions (%)			
		Q<0.6	Q≥0.6	Q≥0.8	Q≥0.9
Jujube	85	4 (4.7%)	81 (95.3%)	68 (80.0%)	53 (62.4%)
Sour Jujube	55	13 (23.6%)	42 (76.4%)	37 (67.3%)	28 (50.9%)
Total	140	17 (12.1%)	123 (87.9%)	105 (75.0%)	81 (57.9%)

Morphological, biological, and cytological evidence indicates that sour jujube is a wild species of jujube and that jujube is derived from sour jujube. Zhang et al. (2015b) used seven SSR makers to classify 17 sour jujubes and 16 jujube varieties into wild, semi-wild, and cultivar species, with frequent genetic exchanges observed among the three groups. Huang et al. (2015) used chloroplast microsatellite (cpSSR) markers to analyze jujube, sour jujube, and Indian jujube. The results also showed that a genetic exchange existed between sour jujube and jujube. In this study, the cluster analysis showed that jujube and sour jujube were obviously divided into two group, but some of the sour jujube individuals had a closer genetic relationship with the jujube cultivars. Therefore, we divided the 140 samples into wild, semi-wild, and cultivar species (Figure 2). Population structure analysis showed that there was gene flow between the sour jujube and jujube varieties (Figure 4). Our results validated previous research results and provided molecular biological evidence for the cultivation of jujube from sour jujube.

Previous studies have shown that the genetic relationships between different jujube varieties correlate, to an extent, with the origin of the variety (Liu et al., 2016). The genetic variation of jujube mainly emanates from intra-population variation, and the contribution rate from among-population variation is low. Among the 85 jujube varieties used in this study, four accessions with a Q-value of less than 0.6 accounted for only 4.7%, and most of the species had a single kinship (Q≥0.8), which indicated that the majority of the varieties are dominated by intra-population or intra-geographic variation (Table 6). The above results indicate that the existing germplasm resources of jujube may originate from different regions. Frequent gene exchange and recombination have occurred among the intraspecific cultivars during the evolution of the species, resulting in a more varied population structure composition.

Genome sequencing showed that the jujube genome contains a very high density of SSRs. The SSR repeats exhibited a strong bias toward A/T, AT/TA, and AAT/ATT motifs, whereas C/G and CG/CG motifs were present at very low levels (Xiao et al., 2015; Fu et al., 2016). Interestingly, the analysis of SSR and ISSR markers showed that AG/GA, CT/TC, and AC/CA repeat motifs had high amplification efficiency, while A/T, AT/TA, and AAT/ATT repeat motifs had low amplification efficiency. The SSRs in our study included 10 AG/GA-, eight CT/TC-, four GT/TG-, and three AC-type primers, which respectively corresponded to 35.7%, 28.6%, 14.3%, and 10.7% of the total SSRs (Table 1). The above results indicate that the simple sequence repeats in the jujube genome are dominated by A/T, AT/TA, and AAT/ATT repeat motifs, but the polymorphic sites are mainly AG/GA, CT/TC, and AC/CA repeat motifs. Therefore, using AG/GA, CT/TC, and AC/CA repeats in primer design could greatly improve primer screening efficiency. This should inform future genetic diversity analyses and the molecular breeding of jujube.

3 Conclusions

In this study, 42 polymorphic alleles were revealed with 28 ISSR primers, each primer amplified polymorphic loci ranged from 1 to 3, with an average of 1.5 for each primer pairs. PIC values for the primer pairs ranged from 0.168 to 0.777. By comprehensive analysis of the genetic diversity and population structure, jujube and sour jujube accessions were roughly divided into two subgroups and most jujube cultivars have a weak correlation with their origin. These results will provide reliable and efficient genetic information for the study of jujube genetic relationship and new variety selection.

Table 6 Summary of primers used in this study

Primer name	Repeat motifs	Sequence 5'–3'	Annealing temperature (°C)
ISSR-1	AT	ATATATATATATATATT	40
ISSR-2	AT	ATATATATATATATATG	40
ISSR-3	AT	ATATATATATATATATC	40
ISSR-4	TA	TATATATATATATATAA	40
ISSR-5	TA	TATATATATATATATAC	40
ISSR-6	TA	TATATATATATATATAG	40
ISSR-7	AG	AGAGAGAGAGAGAGAGT	50
ISSR-8	AG	AGAGAGAGAGAGAGAGC	50
ISSR-9	AG	AGAGAGAGAGAGAGAGG	50
ISSR-10	GA	GAGAGAGAGAGAGAGAT	50
ISSR-11	GA	GAGAGAGAGAGAGAGAC	50
ISSR-12	GA	GAGAGAGAGAGAGAGAA	50
ISSR-13	CT	CTCTCTCTCTCTCTCTT	50
ISSR-14	CT	CTCTCTCTCTCTCTCTA	50
ISSR-15	CT	CTCTCTCTCTCTCTCTG	50
ISSR-16	CA	CACACACACACACACAT	50
ISSR-17	CA	CACACACACACACACAA	50
ISSR-18	CA	CACACACACACACACAG	50
ISSR-19	GT	GTGTGTGTGTGTGTGTA	50
ISSR-20	GT	GTGTGTGTGTGTGTGTC	50
ISSR-21	GT	GTGTGTGTGTGTGTGTT	50
ISSR-22	TC	TCTCTCTCTCTCTCTCA	50
ISSR-23	TC	TCTCTCTCTCTCTCTCC	50
ISSR-24	TC	TCTCTCTCTCTCTCTCG	50
ISSR-25	AC	ACACACACACACACACT	50
ISSR-26	AC	ACACACACACACACACC	50
ISSR-27	AC	ACACACACACACACACG	50
ISSR-28	TG	TGTGTGTGTGTGTGTGA	50
ISSR-29	TG	TGTGTGTGTGTGTGTGC	50
ISSR-30	TG	TGTGTGTGTGTGTGTGG	50
ISSR-31	AT	ATATATATATATATATTA	40
ISSR-32	AT	ATATATATATATATATGA	40
ISSR-33	AT	ATATATATATATATATCA	40
ISSR-34	AT	ATATATATATATATATTC	40
ISSR-35	AT	ATATATATATATATATGC	40
ISSR-36	AT	ATATATATATATATATCC	40
ISSR-37	AT	ATATATATATATATATTG	40
ISSR-38	AT	ATATATATATATATATGG	40
ISSR-39	AT	ATATATATATATATATCG	40
ISSR-40	AG	AGAGAGAGAGAGAGAGTT	55
ISSR-41	AG	AGAGAGAGAGAGAGAGGT	55
ISSR-42	AG	AGAGAGAGAGAGAGAGCT	55
ISSR-43	AG	AGAGAGAGAGAGAGAGTC	55
ISSR-44	AG	AGAGAGAGAGAGAGAGGC	55
ISSR-45	AG	AGAGAGAGAGAGAGAGCC	55
ISSR-46	AG	AGAGAGAGAGAGAGAGTA	55
ISSR-47	AG	AGAGAGAGAGAGAGAGGA	55

Continuing Table 6

Primer name	Repeat motifs	Sequence 5'–3'	Annealing temperature (°C)
ISSR-48	AG	AGAGAGAGAGAGAGAGCA	55
ISSR-49	TA	TATATATATATATATAAT	50
ISSR-50	TA	TATATATATATATATAGT	50
ISSR-51	TA	TATATATATATATATAAC	50
ISSR-52	TA	TATATATATATATATAGC	50
ISSR-53	TA	TATATATATATATATAAG	50
ISSR-54	TA	TATATATATATATATAGG	50
ISSR-55	GA	GAGAGAGAGAGAGAGATT	55
ISSR-56	GA	GAGAGAGAGAGAGAGAGT	55
ISSR-57	GA	GAGAGAGAGAGAGAGACT	55
ISSR-58	GA	GAGAGAGAGAGAGAGATC	55
ISSR-59	GA	GAGAGAGAGAGAGAGAGC	55
ISSR-60	GA	GAGAGAGAGAGAGAGACC	55
ISSR-61	GA	GAGAGAGAGAGAGAGATG	55
ISSR-62	GA	GAGAGAGAGAGAGAGAGG	55
ISSR-63	GA	GAGAGAGAGAGAGAGACG	55
ISSR-64	CT	CTCTCTCTCTCTCTCTAA	55
ISSR-65	CT	CTCTCTCTCTCTCTCTGA	55
ISSR-66	CT	CTCTCTCTCTCTCTCTAC	55
ISSR-67	CT	CTCTCTCTCTCTCTCTGC	55
ISSR-68	CT	CTCTCTCTCTCTCTCTAG	55
ISSR-69	CT	CTCTCTCTCTCTCTCTGG	55
ISSR-70	CA	CACACACACACACACAAT	55
ISSR-71	CA	CACACACACACACACAGT	55
ISSR-72	CA	CACACACACACACACAAC	55
ISSR-73	CA	CACACACACACACACAGC	55
ISSR-74	CA	CACACACACACACACAAG	55
ISSR-75	CA	CACACACACACACACAGG	55
ISSR-76	GT	GTGTGTGTGTGTGTGTTA	55
ISSR-77	GT	GTGTGTGTGTGTGTGTGA	55
ISSR-78	GT	GTGTGTGTGTGTGTGTCA	55
ISSR-79	GT	GTGTGTGTGTGTGTGTTC	55
ISSR-80	GT	GTGTGTGTGTGTGTGTGC	55
ISSR-81	GT	GTGTGTGTGTGTGTGTCC	55
ISSR-82	GT	GTGTGTGTGTGTGTGTTG	55
ISSR-83	GT	GTGTGTGTGTGTGTGTGG	55
ISSR-84	GT	GTGTGTGTGTGTGTGTCG	55
ISSR-85	TC	TCTCTCTCTCTCTCTCAA	55
ISSR-86	TC	TCTCTCTCTCTCTCTCGA	55
ISSR-87	TC	TCTCTCTCTCTCTCTCAT	55
ISSR-88	TC	TCTCTCTCTCTCTCTCGT	55
ISSR-89	TC	TCTCTCTCTCTCTCTCAG	55
ISSR-90	TC	TCTCTCTCTCTCTCTCGG	55
ISSR-91	AC	ACACACACACACACACTT	55
ISSR-92	AC	ACACACACACACACACGT	55
ISSR-93	AC	ACACACACACACACACCT	55
ISSR-94	AC	ACACACACACACACACTA	55

Continuing Table 6

Primer name	Repeat motifs	Sequence 5'–3'	Annealing temperature (°C)
ISSR-95	AC	ACACACACACACACACGA	55
ISSR-96	AC	ACACACACACACACACCA	55
ISSR-97	AC	ACACACACACACACACTG	55
ISSR-98	AC	ACACACACACACACACGG	55
ISSR-99	AC	ACACACACACACACACCG	55
ISSR-100	TG	TGTGTGTGTGTGTGTGAT	55
ISSR-101	TG	TGTGTGTGTGTGTGTGGT	55
ISSR-102	TG	TGTGTGTGTGTGTGTGAC	55
ISSR-103	TG	TGTGTGTGTGTGTGTGGC	55
ISSR-104	TG	TGTGTGTGTGTGTGTGAA	55
ISSR-105	TG	TGTGTGTGTGTGTGTGGA	55
ISSR-106	AC	ACCACCACCACCACCACC	60
ISSR-107	AGC	AGCAGCAGCAGCAGCAGC	60
ISSR-108	AGT	AGTAGTAGTAGTAGTAGT	50
ISSR-109	ATG	ATGATGATGATGATGATG	50
ISSR-110	CCG	CCGCCGCCGCCGCCGCCG	60
ISSR-111	CTC	CTCCTCCTCCTCCTCCTC	60
ISSR-112	GGC	GGCGGCGGCGGCGGCGGC	60
ISSR-113	GAA	GAAGAAGAAGAAGAAGAA	50
ISSR-114	GTT	GTTGTTGTTGTTGTTGTT	50
ISSR-115	TGC	TGCTGCTGCTGCTGCTGC	60
ISSR-116	TAT	TATTATTATTATTATTAT	50
ISSR-117	GATA	GATAGATAGATAGATA	50
ISSR-118	GACA	GACAGACAGACAGACA	50
ISSR-119	CCCT	CCCTCCCTCCCTCCCT	55
ISSR-120	CTAG	CTAGCTAGCTAGCTAG	50
ISSR-121	GATA	GATAGATAGACAGACA	50
ISSR-122	TGCA	TGCATGCATGCATGCA	50
ISSR-123	GGAT	GGATGGATGGATGGAT	50
ISSR-124	CTTCA	CTTCACTTCACTTCA	50
ISSR-125	GA	GGAGAGGAGAGGAGA	50
ISSR-126	GGGT	GGGTGGGGTGGGGTG	55
ISSR-127	AT	ACAATATATATATATAT	50
ISSR-128	AT	CCCATATATATATATAT	50
ISSR-129	AT	GCGATATATATATATAT	50
ISSR-130	AT	AGAATATATATATATAT	50
ISSR-131	AT	CGCATATATATATATAT	50
ISSR-132	AT	CTCATATATATATATAT	50
ISSR-133	AT	GCGATATATATATATAT	50
ISSR-134	AT	GGGATATATATATATAT	50
ISSR-135	AT	GTGATATATATATATAT	50
ISSR-136	TA	CACTATATATATATATA	50
ISSR-137	TA	CCCTATATATATATATA	50
ISSR-138	TA	CGCTATATATATATATA	50
ISSR-139	TA	GAGTATATATATATATA	50
ISSR-140	TA	GCGTATATATATATATA	50
ISSR-141	TA	GGGTATATATATATATA	50

Continuing Table 6

Primer name	Repeat motifs	Sequence 5'–3'	Annealing temperature (°C)
ISSR-142	TA	TATTATATATATATATA	50
ISSR-143	TA	TCTTATATATATATATA	50
ISSR-144	TA	TGTTATATATATATATA	50
ISSR-145	AG	ACAAGAGAGAGAGAGAG	50
ISSR-146	AG	AGAAGAGAGAGAGAGAG	50
ISSR-147	AG	ATAAGAGAGAGAGAGAG	50
ISSR-148	AG	CCCAGAGAGAGAGAGAG	55
ISSR-149	AG	CGCAGAGAGAGAGAGAG	55
ISSR-150	AG	CTCAGAGAGAGAGAGAG	50
ISSR-151	AG	TCTAGAGAGAGAGAGAG	50
ISSR-152	AG	TGTAGAGAGAGAGAGAG	50
ISSR-153	AG	TTTAGAGAGAGAGAGAG	50
ISSR-154	GA	CACGAGAGAGAGAGAGA	50
ISSR-155	GA	CCCGAGAGAGAGAGAGA	55
ISSR-156	GA	CTCGAGAGAGAGAGAGA	50
ISSR-157	GA	GAGGAGAGAGAGAGAGA	50
ISSR-158	GA	GCGGAGAGAGAGAGAGA	55
ISSR-159	GA	GTGGAGAGAGAGAGAGA	50
ISSR-160	GA	TATGAGAGAGAGAGAGA	50
ISSR-161	GA	TCTGAGAGAGAGAGAGA	50
ISSR-162	GA	TTTGAGAGAGAGAGAGA	50
ISSR-163	CT	AAACTCTCTCTCTCTCT	50
ISSR-164	CT	AGACTCTCTCTCTCTCT	50
ISSR-165	CT	ATACTCTCTCTCTCTCT	50
ISSR-166	CT	CACCTCTCTCTCTCTCT	50
ISSR-167	CT	CGCCTCTCTCTCTCTCT	55
ISSR-168	CT	CTCCTCTCTCTCTCTCT	50
ISSR-169	CT	GAGCTCTCTCTCTCTCT	50
ISSR-170	CT	GGGCTCTCTCTCTCTCT	55
ISSR-171	CT	GTGCTCTCTCTCTCTCT	50
ISSR-172	TC	AAATCTCTCTCTCTCTC	50
ISSR-173	TC	ACATCTCTCTCTCTCTC	50
ISSR-174	TC	AGATCTCTCTCTCTCTC	50
ISSR-175	TC	GAGTCTCTCTCTCTCTC	50
ISSR-176	TC	GCGTCTCTCTCTCTCTC	55
ISSR-177	TC	GGGTCTCTCTCTCTCTC	55
ISSR-178	TC	TATTCTCTCTCTCTCTC	50
ISSR-179	TC	TCTTCTCTCTCTCTCTC	50
ISSR-180	TC	TGTTCTCTCTCTCTCTC	50
ISSR-181	CA	CACCACACACACACACA	50
ISSR-182	CA	CGCCACACACACACACA	55
ISSR-183	CA	CTCCACACACACACACA	50
ISSR-184	CA	GAGCACACACACACACA	50
ISSR-185	CA	GGGCACACACACACACA	55
ISSR-186	CA	GTGCACACACACACACA	50
ISSR-187	CA	TATCACACACACACACA	50
ISSR-188	CA	TGTCACACACACACACA	50

Continuing Table 6

Primer name	Repeat motifs	Sequence 5'–3'	Annealing temperature (°C)
ISSR-189	CA	TTTCACACACACACACA	50
ISSR-190	AC	ACAACACACACACACAC	50
ISSR-191	AC	AGAACACACACACACAC	50
ISSR-192	AC	ATAACACACACACACAC	50
ISSR-193	AC	GCGACACACACACACAC	55
ISSR-194	AC	GGGACACACACACACAC	55
ISSR-195	AC	GTGACACACACACACAC	50
ISSR-196	AC	TCTACACACACACACAC	50
ISSR-197	AC	TGTACACACACACACAC	50
ISSR-198	AC	TTTACACACACACACAC	50
ISSR-199	GT	AAAGTGTGTGTGTGTGT	50
ISSR-200	GT	ACAGTGTGTGTGTGTGT	50
ISSR-201	GT	ATAGTGTGTGTGTGTGT	50
ISSR-202	GT	CACGTGTGTGTGTGTGT	50
ISSR-203	GT	CCCGTGTGTGTGTGTGT	55
ISSR-204	GT	CTCGTGTGTGTGTGTGT	50
ISSR-205	GT	GAGGTGTGTGTGTGTGT	50
ISSR-206	GT	GCGGTGTGTGTGTGTGT	55
ISSR-207	GT	GTGGTGTGTGTGTGTGT	50
ISSR-208	TG	AAATGTGTGTGTGTGTG	50
ISSR-209	TG	ACATGTGTGTGTGTGTG	50
ISSR-210	TG	AGATGTGTGTGTGTGTG	50
ISSR-211	TG	CACTGTGTGTGTGTGTG	50
ISSR-212	TG	CCCTGTGTGTGTGTGTG	55
ISSR-213	TG	CGCTGTGTGTGTGTGTG	55
ISSR-214	TG	TATTGTGTGTGTGTGTG	50
ISSR-215	TG	TCTTGTGTGTGTGTGTG	50
ISSR-216	TG	TGTTGTGTGTGTGTGTG	50

4 Materials and Methods

4.1 Plant materials

In total, 140 samples included 85 cultivars from Chinese jujube and 55 individuals from sour jujube (Table 2). These materials maintained in jujube germplasm resources of Luoyang Normal University (Luoyang, Henan) were acquired with permissions from the National Chinese Jujube Germplasm Repository (Taigu, Shanxi), the National Foundation for Improved Cultivar of Chinese Jujube (Cangzhou, Hebei) and the Xinzheng Jujube Academy of Science (Xinzheng, Henan). Fresh young leaves for each accession were collected in May 2017, brought to the laboratory in an ice box, and stored in -70°C freezer till further analysis.

4.2 Genomic DNA extraction and PCR analysis

Genomic DNA was extracted using a modified CTAB method (Lian et al., 2006). The DNA quality was assessed using a NanoDrop2000 and the DNA was diluted to 50 ng/μL. Sequences of 216 ISSR primers were obtained from the Biotechnology Laboratory at the University of British Columbia (Vancouver, Canada; Table 7). Polymerase chain reaction (PCR) amplification was performed in a 10 μL reaction mixture containing 2.0 μL of template DNA, 0.4 μL of primers (10 μM), 0.8 μL of dNTP (2.5 mM), 1.0 μL of 10×Buffer, 0.2 μL of Taq DNA Polymerase (Solarbio, Beijing, China), and 5.6 μL of deionized water. PCR amplifications were performed in 96-well plates on a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: 94°C for 3 min; 35 cycles at 94°C for 30 s, 40°C~60°C (melting temperature depends on the primer sets as listed in Table 7) for 30 s, and 72°C for 1.5 min; and a final extension at 72°C for 10 min. The amplified products were separated by electrophoresis on 2.0% (w/v) agarose gels under UV light.

Table 7 Inferred ancestry of the 140 accessions based on Bayesian analysis

Accessions	Inferred clusters	
	Cluster I	Cluster II
J01	0.7716	0.2284
J02	0.9756	0.0244
J03	0.6077	0.3923
J04	0.4955	0.5045
J05	0.982	0.018
J06	0.5183	0.4817
J07	0.87	0.13
J08	0.9833	0.0167
J09	0.7421	0.2579
J10	0.9447	0.0553
J11	0.8421	0.1579
J12	0.979	0.021
J13	0.979	0.021
J14	0.8884	0.1116
J15	0.8858	0.1142
J16	0.8054	0.1946
J17	0.8631	0.1369
J18	0.9809	0.0191
J19	0.8961	0.1039
J20	0.842	0.158
J21	0.5886	0.4114
J22	0.8604	0.1396
J23	0.6531	0.3469
J24	0.9209	0.0791
J25	0.773	0.227
J26	0.9861	0.0139
J27	0.9462	0.0538
J28	0.9813	0.0187
J29	0.9682	0.0318
J30	0.9512	0.0488
J31	0.986	0.014
J32	0.9702	0.0298
J33	0.9334	0.0666
J34	0.9384	0.0616
J35	0.9841	0.0159
J36	0.9802	0.0198
J37	0.8852	0.1148
J38	0.7261	0.2739
J39	0.9344	0.0656
J40	0.6803	0.3197
J41	0.9831	0.0169
J42	0.984	0.016
J43	0.9762	0.0238
J44	0.9533	0.0467
J45	0.7967	0.2033
J46	0.9815	0.0185
J47	0.7419	0.2581
J48	0.9832	0.0168
J49	0.9765	0.0235
J50	0.9675	0.0325
J51	0.9852	0.0148

Continuing Table 7

Accessions	Inferred clusters	
	Cluster I	Cluster II
J52	0.9629	0.0371
J53	0.9782	0.0218
J54	0.7965	0.2035
J55	0.9742	0.0258
J56	0.985	0.015
J57	0.987	0.013
J58	0.9662	0.0338
J59	0.968	0.032
J60	0.9769	0.0231
J61	0.9581	0.0419
J62	0.9802	0.0198
J63	0.7295	0.2705
J64	0.9393	0.0607
J65	0.9692	0.0308
J66	0.9614	0.0386
J67	0.9879	0.0121
J68	0.9793	0.0207
J69	0.9832	0.0168
J70	0.988	0.012
J71	0.985	0.015
J72	0.9501	0.0499
J73	0.988	0.012
J74	0.9354	0.0646
J75	0.983	0.017
J76	0.8357	0.1643
J77	0.8757	0.1243
J78	0.471	0.529
J79	0.9142	0.0858
J80	0.8405	0.1595
J81	0.8386	0.1614
J82	0.9499	0.0501
J83	0.7207	0.2793
J84	0.8029	0.1971
J85	0.7338	0.2662
S01	0.3805	0.6195
S02	0.4215	0.5785
S03	0.9669	0.0331
S04	0.6386	0.3614
S05	0.0573	0.9427
S06	0.3189	0.6811
S07	0.0241	0.9759
S08	0.0538	0.9462
S09	0.3233	0.6767
S10	0.6448	0.3552
S11	0.1037	0.8963
S12	0.0794	0.9206
S13	0.0346	0.9654
S14	0.1483	0.8517
S15	0.1176	0.8824
S16	0.0226	0.9774
S17	0.0548	0.9452

Continuing Table 7

Accessions	Inferred clusters	
	Cluster I	Cluster II
S18	0.0399	0.9601
S19	0.0572	0.9428
S20	0.0361	0.9639
S21	0.4628	0.5372
S22	0.025	0.975
S23	0.0469	0.9531
S24	0.0292	0.9708
S25	0.2675	0.7325
S26	0.1223	0.8777
S27	0.019	0.981
S28	0.1154	0.8846
S29	0.0443	0.9557
S30	0.4695	0.5305
S31	0.0251	0.9749
S32	0.1043	0.8957
S33	0.0199	0.9801
S34	0.0885	0.9115
S35	0.0576	0.9424
S36	0.0338	0.9662
S37	0.0464	0.9536
S38	0.1207	0.8793
S39	0.0368	0.9632
S40	0.1017	0.8983
S41	0.018	0.982
S42	0.026	0.974
S43	0.027	0.973
S44	0.0952	0.9048
S45	0.0296	0.9704
S46	0.8219	0.1781
S47	0.7542	0.2458
S48	0.7218	0.2782
S49	0.0154	0.9846
S50	0.6563	0.3437
S51	0.7359	0.2641
S52	0.3842	0.6158
S53	0.4102	0.5898
S54	0.9776	0.0224
S55	0.1776	0.8224

4.3 Genetic diversity analysis

Based on the relative position of the ISSR amplification product on the agarose gel, the presence and absence of bands at the same position were scored as "1" and "0", respectively. The following parameters were calculated using GenALEx 6.5 (Peakall and Smouse, 2012): the number of different alleles (Na), the effective number of alleles (Ne), the Shannon index (I), and the polymorphic information content (PIC).

The cluster analysis was performed using the sequential, agglomerative, hierarchical, and nested clustering (SAHN) module and the unweighted pair-group method arithmetic average (UPGMA) method of NTSYS-pc2.10e software, and a cluster plot was generated by the Tree plot module (Rohlf, 1998).

4.4 Population structure analysis

A Bayesian clustering analysis was implemented in Structure 2.3.4 (Hubisz et al., 2009) to evaluate population genetic structure. An admixture model and correlated allele frequencies were applied to estimate the ancestry fractions of each cluster attributed to each accession. For each value of K (range 1-25), 10 independent runs were performed with a burn-in period of 100,000 followed by 1,000,000 MCMC repetitions. Parameters were set to default values, and all accessions were considered to have unknown origins. The delta K method (Evanno et al., 2005) was implemented in Structure Harvester program (Earl and Vonholdt, 2012) to determine the most probable K-value. The accessions with membership probabilities ≥ 0.50 were considered to belong to the same group (Chen et al., 2017b). A principal coordinate analysis (PCoA) and Mantel test were performed using GenAlEx v 6.5.

Authors' contributions

S.P.L. and M.X.G. conducted most of the experiments, including germplasm collection, genomic DNA extraction and PCR analysis, genetic diversity and cluster analysis, and population structure analysis. S.T. participated in germplasm collection and DNA extraction. H.X.L. participated in germplasm collection. X.S.Z. initiated the project. S.P.L. and X.S.Z. designed and supervised the study. S.P.L. and M.X.G. analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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