Assessment of Genetic Diversity of Chinese Sand Pear Landraces (*Pyrus pyrifolia* Nakai) Using Simple Sequence Repeat Markers

Zhengwang Jiang and Feiyan Tang

Wuhan Botanical Garden/Wuhan Institute of Botany, Chinese Academy of Sciences, Wuhan, Hubei 430074, China

Hongwen Huang¹

South China Botanical Garden/South China Institute of Botany, Chinese Academy of Sciences, Guangzhou, Guangdong 510650, China

Hongju Hu and Qiliang Chen

Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences, Wuhan, Hubei 430209, China

Additional index words. *Pyrus pyrifolia*, microsatellites, landrace, genetic diversity, sand pear germplasm

Abstract. The sand pear (*Pyrus pyrifolia* Nakai) is an important fruit crop in China. In this study, simple sequence repeats (SSRs) were used to estimate the level and pattern of genetic diversity among 233 sand pear landraces collected from 10 different geographic regions in China. The results demonstrated that the SSR technique is an effective tool for assessing genetic diversity and the geographic pattern of genetic variation among sand pear landraces of different origins. A total of 184 putative alleles was detected using 14 primer pairs with an average of 13.1 alleles per locus. The mean expected heterozygosity and observed heterozygosity across all loci were 0.705 and 0.671, respectively. High genetic diversity was found in all populations except for that originated from Jiangxi ($A_e = 3.149$; $H_e = 0.655$), whereas at the regional level, those from central China were less diverse than those from other regions. Analysis of molecular variance showed that most genetic differences resided among landraces within populations. Additionally, unweighted pair group with arithmetic average clustering and principal component analysis plotting based on Nei's genetic distance revealed distinct gene pools in agreement with geographic distribution.

The sand pear (*Pyrus pyrifolia* Nakai) is one of the most important fruit tree crops in China and is extensively cultivated in central and southwest China. The species occurs naturally in southern and western China, recognized as the center of origin of the genus *Pyrus* (Rubtsov, 1944). There are very many landraces (local cultivars) of *P. pyrifolia* owing to nearly 3000 years of cultivation and the complex climatic and geographical variation in China. Many landraces have unique traits. For example, 'Puguali', from Zhejiang Province, is a large-fruited cultivar with a mean weight of 553 g and a maximum weight of 950 g. The skin is green when mature and covered with brown russet, which turns reddish brown when fruit are fully ripe. 'Cangxili', named after its place of origin, Cangxi county in Sichuan Province, is a traditional landrace

with maximum fruit weight of 1850 g (average, 321.3 g), smooth skin, and crisp and tender flesh, which is sweet and juicy and of high quality. The rich genetic resources in sand pear provide great potential for cultivar improvement and enhancement of the sustainability of the pear industry. However, many traditional local cultivars have been threatened with extinction by the changes that have occurred in the modern Chinese fruit industry over the past three decades. There has been large-scale cultivation of a few elite cultivars and top-grafting or replacement of old cultivars or landraces. This genetic loss could lead to serious erosion of the gene pool of the cultivated sand pear. To conserve and manage the diversity of sand pear landraces and cultivars, the Wuhan Sand Pear Germplasm Repository (WSPGR) was established in 1986 as the national repository for sand pears, and since then, an exhaustive collection of local cultivars and landraces of Chinese sand pear has been assembled.

Molecular techniques are useful tools for evaluating genetic diversity and for defining genetic relationships in fruit tree crops. In pear, chloroplast polymerase chain reaction–restriction fragment length polymorphisms

(RFLPs) were used to examine relationships between east Asian species (Iketani et al., 1998). Dominant nuclear markers, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and intersimple sequence repeat (ISSR) were used in an investigation of genetic relationships among species and for pear cultivar fingerprinting (Cao et al., 2007; Shen et al., 2006; Teng et al., 2001, 2002; Zhang et al., 2007), and nuclear and chloroplast DNA sequences have been used to identify pear cultivars (Kimura et al., 2003; Lee et al., 2004). Of the DNA marker systems currently available, simple sequence repeats (SSRs) have been considered one of the most useful for assessment of genetic diversity and cultivar fingerprinting at the intraspecific level because of their abundance, hyperpolymorphism, and codominant inheritance (Morgante and Olivieri, 1993; Tautz, 1989). However, the published work to date reported the use of a set of SSRs isolated from apple for verifying the transferability of SSRs between apple and pear (Yamamoto et al., 2001) and the cultivar identification by SSRs in a limited number of cultivars developed in Japan (Kimura et al., 2002). SSR markers have been proved as a robust tool for revealing genetic diversity in sand pear (Cao et al., 2007; Kimura et al., 2002, 2003), red-skin sand pear (Zhang et al., 2007), and some other pears in west China (Fan et al., 2007).

Current germplasm evaluation in China mostly focuses on morphological descriptions and documenting pomological traits. The exchange of plants between repositories or commercial orchards raises problems in that some individual sand pear landraces or cultivars may be known by several different names or the one name may be used for different landraces or cultivars. The information from current evaluation of sand pears was not sufficient. Furthermore, detailed morphological descriptions and comparisons of plants are time-consuming and fruit-related traits cannot be observed until plants are mature to produce fruit. Genetic characterization of the gene pool of the cultivated sand pears in the WSPGR collection has not previously been attempted but is urgently needed for formulating management strategies for the WSPGR and for furnishing useful genetic information for future sand pear breeding efforts. This should provide a better understanding of the genetic diversity that exists in the gene pool of the cultivated sand pears and the diverse sources of useful genes in the germplasm repository. Therefore, the objectives of the present investigation were to determine the genetic diversity of the overall gene pool of sand pear landraces and assess the genetic variation among sand pear landrace groups in relation to their geographical distribution in China.

Materials and Methods

A total of 233 landraces originating from 10 provinces (designated as populations in this study) was obtained from the WSPGR

Received for publication 2 Oct. 2007. Accepted for publication 6 Jan. 2008.

This research was supported by the CAS project KSCX2-YW-N-061 and NSFC grant 30070082.

¹To whom reprint requests should be addressed; e-mail huanghw@mail.scbg.ac.cn.

Table 1. Chinese landraces of sand pear evaluated.

Accession number	Landrace	Original place of collection		
		District	Province (population)	Region
001	Fu'andaxueli	Fu'an	Fujian	East China
002	Qingpizhongli	Jian'ou	Fujian	East China
003	Aijiali	Jianyang	Fujian	East China
004	Bingzili	Jianyang	Fujian	East China
005	Huangpizhongli	Jianyang	Fujian	East China
006	Chipili	Jinjiang	Fujian	East China
007	Eli	Jinjiang	Fujian	East China
008	Pingguoli	Jinjiang	Fujian	East China
009	Shuili	Jinjiang	Fujian	East China
010	Bannannvli	Pingnan	Fujian	East China
011	Mandingxueli	Pucheng	Fujian	East China
012	Puchengxueli	Pucheng	Fujian	East China
013	Panli	Shouning	Fujian	East China
014	Liuyuehuangzongli	Shunchang	Fujian	East China
015	Mali	Shunchang	Fujian	East China
016	Baiyu	Jiujiang	Jiangxi	East China
017	Hehua	Shangrao	Jiangxi	East China
018	Huangpixiao	Shangrao	Jiangxi	East China
019	Kuixingmake	Shangrao	Jiangxi	East China
020	Xihuapingtouqing	Shangrao	Jiangxi	East China
021	Jiangwanxipili	Wuyuan	Jiangxi	East China
022	Bayuexue	Wuyuan	Jiangxi	East China
023	Wuyuanbaili	Wuyuan	Jiangxi	East China
024	Wuyuansuli	Wuyuan	Jiangxi	East China
025	Wuyanxueli	Wuyuan	Jiangxi	East China
026	Yousu	Wuyuan	Jiangxi	East China
027	Danenli	Leqing	Zhejiang	East China
028	Dahuangren	Leqing	Zhejiang	East China
029	Huangqieli	Leqing	Zhejiang	East China
030	Jiuzhongli	Leqing	Zhejiang	East China
031	Puguali	Leqing	Zhejiang	East China
032	Yandangxueli	Leqing	Zhejiang	East China
033	Zhenxiangli	Lishui	Zhejiang	East China
034	Hanghong	Unknown	Zhejiang	East China
035	Huahong	Unknown	Zhejiang	East China
036	Yuanli	Unknown	Zhejiang	East China
037	Ruanxueli	Unknown	Zhejiang	East China
038	Sanhua	Unknown	Zhejiang	East China
039	Shanghaixueli	Unknown	Zhejiang	East China
040	Nuodaoli	Yiwu	Zhejiang	East China
041	Yiwulizi	Yiwu	Zhejiang	East China
042	Zaosanhua	Yiwu	Zhejiang	East China
043	Xihuaxueli	Yunhe	Zhejiang	East China
044	Yunheshibianli	Yunhe	Zhejiang	East China
045	Yunhexueli	Yunhe	Zhejiang	East China
046	Fengkaihuizouli	Fengkai	Guangdong	South China
047	Xinghuadayinli	Fengkai	Guangdong	South China
048	Yeshenli	Fengkai	Guangdong	South China
049	Hehuali	Fengkai	Guangdong	South China
050	Gaoyadanshuili	Gaoyao	Guangdong	South China
051	Gaoyaohuangli	Gaoyao	Guangdong	South China
052	Gaoyaoqingli	Gaoyao	Guangdong	South China
053	Jianyeli	Gaoyao	Guangdong	South China
054	Huiyangqingli	Huiyang	Guangdong	South China
055	Huiyangsuanli	Huiyang	Guangdong	South China
056	Xihuahongli	Huiyang	Guangdong	South China
057	Xiangshuili	Huiyang	Guangdong	South China
058	Qingpili	Lianping	Guangdong	South China
059	Sianqingpili	Baise	Guangxi	South China
060	Beiliuhuangli	Beiliu	Guangxi	South China
061	Beiliumili	Beiliu	Guangxi	South China
062	Beiliuqingli	Beiliu	Guangxi	South China
063	Xiangjiaoli	Debao	Guangxi	South China
064	Huangpichangbatangli	Gongcheng	Guangxi	South China
065	Huangpisuanli	Gongcheng	Guangxi	South China
066	Huangpixueli	Gongcheng	Guangxi	South China
067	Qingpisuanli	Gongcheng	Guangxi	South China
068	Xipitangli	Gongcheng	Guangxi	South China
069	Cupitangli	Guanyang	Guangxi	South China
070	Guanyanghongli	Guanyang	Guangxi	South China
071	Guanyangqingshuili	Guanyang	Guangxi	South China
072	Guanyangshuanli	Guanyang	Guangxi	South China

(Continued on next page)

(Wuhan, China). Provinces were assigned to four regions (east, south, central, and southwest China). Details are given in Table 1 and Figure 1, and the numbers of landraces for each region and population are shown in Table 2.

Total genomic DNA was extracted from 1 to 2 g of fresh leaf tissue using the 2 × CTAB method of Doyle and Doyle (1987) with a slight modification (3 × CTAB). Extracted DNA was quantified in a BioPhotometer spectrophotometer (Eppendorf, Hamburg, Germany).

Sixteen SSR primer pairs developed from sand pear (*P. pyrifolia*) (Yamamoto et al., 2002b) and two developed from apple (Gianfranceschi et al., 1998) were used for initial assay and of these, 14 SSR primer pairs were chosen based on gel clarity and reliability of alleles (Table 3). Polymerase chain reactions were performed in a final volume of 10 µL containing 20 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, 0.1% Tween 20, 75 mM Tris-HCl (pH 8.8), 0.2 mM dNTPs, 0.2 µM of primer, 20 ng genomic DNA, and 0.5 unit Taq polymerase (Fermentas, Lithuania). The amplification was carried out in a PTC-200 thermal cycler (MJ Research, Watertown, MA) with the following protocol: 5 min at 95 °C followed by 35 cycles of 50 s at 94 °C, 50 s at 55 °C, and 50 s at 72 °C followed by a final 8-min extension at 72 °C. The amplification products with an aliquot 25 bp DNA ladder (Promega, Madison, WI) were electrophoresed in 6% denaturing polyacrylamide gel using a Sequi-Gen® GT/Power Pac 3000 Sequencer System (Bio-Rad, Hercules, CA) for ≈1.5 h at 55 W followed by a modified silver staining procedure (Sanguinetti et al., 1994). The bands of amplified DNA fragments were scored manually.

SSR markers were designated by the developer's primer code (referred to as locus) corresponding to the primer pair sequences followed by the product size (bp). Genetic diversity parameters were estimated: number of alleles per locus (*A*), number of effective alleles per locus (*A_e*), observed heterozygosity (*H_o*), expected heterozygosity (*H_e*), and Shannon-Weaver's information index (*I*) using the program GenAlEx (Peakall and Smouse, 2001). The relative magnitude of genetic differentiation between geographic cultivar groups [Wright's *F*-statistic (*F_{ST}*)] was calculated by different methods implemented in analysis of molecular variance (AMOVA) (Excoffier et al., 1992) and GenAlEx software. The power of discrimination ($PD=1 - \sum p_i^2$, where *p_i* is the frequency of the *i*-th genotype (Kloosterman et al., 1993), was also calculated for each locus. Nei et al.'s (1983) genetic distance (*D_A*) between populations was generated by the program POPULATION Version 1.2.28 (Olivier Langella; available at <http://www.pge.cnrs-gif.fr/bioinfo/populations>). *D_A* has been considered the appropriate approach for phylogenetic analysis of closely related populations when SSR markers are used (Takezaki and Nei, 1996). The SAHN procedure of NTSYS-pc2.0 software (Rohlf, 1997) was used to

Table 1. (Continued) Chinese landraces of sand pear evaluated.

Accession number	Landrace	Original place of collection		
		District	Province (population)	Region
073	Guanyangtangli	Guanyang	Guangxi	South China
074	Guanyangxueli	Guanyang	Guangxi	South China
075	Guanyangzaoheli	Guanyang	Guangxi	South China
076	Huangpieli	Guanyang	Guangxi	South China
077	Quanzhouli	Guilin	Guangxi	South China
078	Yanshanhuangpixiao	Guilin	Guangxi	South China
079	Yanshanliuyuxue	Guilin	Guangxi	South China
080	Yanshanqingpixueli	Guilin	Guangxi	South China
081	Hengxianjinpaoli	Hengxian	Guangxi	South China
082	Hengxianlingshanli	Hengxian	Guangxi	South China
083	Hengxianmili	Hengxian	Guangxi	South China
084	Nanningdashali	Hengxian	Guangxi	South China
085	Jingxiqingpili	Jingxi	Guangxi	South China
086	Jingxixueli	Jingxi	Guangxi	South China
087	Shanggangli	Leye	Guangxi	South China
088	Lipuhuangpili	Lipu	Guangxi	South China
089	Lipuxueli	Lipu	Guangxi	South China
090	Liuchengfengshanli	Liucheng	Guangxi	South China
091	Liuchengxueli	Liucheng	Guangxi	South China
092	Sanmenjianghuangli	Liucheng	Guangxi	South China
093	Guihuali	Longsheng	Guangxi	South China
094	Huangcupili	Longsheng	Guangxi	South China
095	Huangxipili	Longsheng	Guangxi	South China
096	Napoqingpili	Tianyang	Guangxi	South China
097	Bingtangli	Unknown	Guangxi	South China
098	Beiliuhuali	Wuming	Guangxi	South China
099	Cangwudashali	Wuzhou	Guangxi	South China
100	Badongjingli	Badong	Hubei	Central China
101	Jianshichengtuoli	Jianshi	Hubei	Central China
102	Jianshichixianfeng	Jianshi	Hubei	Central China
103	Jianshizaoguli	Jianshi	Hubei	Central China
104	Lichuanxiangshui	Lichuan	Hubei	Central China
105	Suizhouzaoshuli	Suizhou	Hubei	Central China
106	Huishuijingai	Unknown	Hubei	Central China
107	Houzuili	Unknown	Hubei	Central China
108	Huangpixiang	Unknown	Hubei	Central China
109	Make	Unknown	Hubei	Central China
110	Zaomili	Wuhan	Hubei	Central China
111	Wanxianfeng	Xianfeng	Hubei	Central China
112	Xianfengbaijie	Xianfeng	Hubei	Central China
113	Xianfengchengtuoli	Xianfeng	Hubei	Central China
114	Xianfenghongjuli	Xianfeng	Hubei	Central China
115	Xianfengxuepingli	Xianfeng	Hubei	Central China
116	Xianfengyangdongli	Xianfeng	Hubei	Central China
117	Xxueping	Xianfeng	Hubei	Central China
118	Xuanenchengtuoli	Xuan'en	Hubei	Central China
119	Xuanenxueli(2)	Xuan'en	Hubei	Central China
120	Xuanenyangdongli	Xuan'en	Hubei	Central China
121	Jinbangtou	Yuan'an	Hubei	Central China
122	Longtuanli	Yuan'an	Hubei	Central China
123	Shilixiang	Yuan'an	Hubei	Central China
124	Suanbiantou	Yuan'an	Hubei	Central China
125	Wangshuibai	Yuan'an	Hubei	Central China
126	Huaihuaxiangshui	Anjiang	Hunan	Central China
127	Baojingyangdong	Baojing	Hunan	Central China
128	Daguoqing	Jinxian	Hunan	Central China
129	Tanghuqing	Jinxian	Hunan	Central China
130	Yadianqing	Jinxian	Hunan	Central China
131	Gengtouqing	Linwu	Hunan	Central China
132	Qingli	Linwu	Hunan	Central China
133	Shexiangli	Linwu	Hunan	Central China
134	Xiangheli	Linwu	Hunan	Central China
135	Xianghuali	Linwu	Hunan	Central China
136	Zamali	Linwu	Hunan	Central China
137	Longhuijuli	Longhui	Hunan	Central China
138	Duanbazao	Yizhang	Hunan	Central China
139	Qingpizao	Yizhang	Hunan	Central China
140	Tianxiaoli	Yizhang	Hunan	Central China
141	Meitanjingai	Meitan	Guizhou	Southwest China
142	Meitanmugua	Meitan	Guizhou	Southwest China
143	Fanli	Weining	Guizhou	Southwest China
144	Weiningbaipili	Weining	Guizhou	Southwest China

(Continued on next page)

construct the unweighted pair group using arithmetic average (UPGMA) dendrogram as described by Sneath and Sokal (1973). A principal coordinate analysis (Gower, 1966) was also conducted using the same software package.

Results

Simple sequence repeat polymorphism and fingerprinting sand pear landraces. Of the 16 primer pairs prescreened, 14 resolved clear SSR banding patterns, whereas those of NH012a were not adequately clear for allele scoring and CH01E12 failed to amplify in several samples. These two primer pairs were therefore discarded. The 14 loci generated a total of 184 alleles among 233 sand pear landraces, varying from six to 21 alleles per locus with an average of 13.1 alleles per locus (Table 3). The mean value of gene diversity (H_e) was 0.705, ranging from 0.527 for NH005b to 0.825 for NH014a. The observed heterozygosity (H_o) ranged from 0.451 for NH013a to 0.844 for NH004a with a mean value 0.671. The fixation index (F), which estimates the degree of allelic fixation by comparison of H_e and H_o , was 0.048, suggesting predominantly outcrossing in sand pears. The mean Wright's F_{ST} was 0.129, ranging from 0.097 to 0.156 (Table 3). The mean estimate of power of discrimination (P_D) was relatively high with mean value of 0.91 and ranged from 0.77 for NH005b to 0.97 for NH014a. These results indicated that the 14 SSR loci used were efficient in distinguishing the 233 sand pear landraces.

A total of 20 unique alleles (alleles present in only one or two landraces) was found in eight populations, ranging from one allele in two landraces from both Jiangxi and Yunnan Provinces to five alleles in five different landraces from Guizhou Province (Table 4). At the geographical region level, southern and central China each has three unique alleles, whereas five and nine were found in eastern China and southwestern China, respectively. In addition, two unique alleles were detected in 'Xianfengbaijie', which came from Hubei Province in central China.

Genetic diversity within and among populations. At the population level, the mean number of alleles per locus (A) ranged from 5.643 in Jiangxi Province to 8.500 in Guangxi and Guizhou, and the mean effective number of alleles per locus (A_e) was also lowest in Jiangxi (3.149) and highest in Guangxi (5.071) (Table 2). The estimates of expected heterozygosity (H_e) and Shannon-Weaver's information index (I) showed gene diversity to be highest in Guangxi ($H_e = 0.773$, $I = 1.765$) and lowest in Jiangxi ($H_e = 0.655$, $I = 1.322$) (Table 4). At the regional level, landraces in central China were found to be genetically less diverse than those in other regions, whereas the eastern, southern, and southwestern China regions had similar levels of genetic diversity (Table 2).

Table 1. (Continued) Chinese landraces of sand pear evaluated.

Accession number	Landrace	Original place of collection		
		District	Province (population)	Region
145	Weiningbaiwali	Weining	Guizhou	Southwest China
146	Weiningchengxiangli	Weining	Guizhou	Southwest China
147	Weinigcili	Weining	Guizhou	Southwest China
148	Weiningdahuangli	Weining	Guizhou	Southwest China
149	Weininghuahong	Weining	Guizhou	Southwest China
150	Weininghuapili	Weining	Guizhou	Southwest China
151	Weininghuazhali	Weining	Guizhou	Southwest China
152	Weininghuangshuili	Weining	Guizhou	Southwest China
153	Weininghuangsuanli	Weining	Guizhou	Southwest China
154	Weiningjingaili	Weining	Guizhou	Southwest China
155	Weiningmali	Weining	Guizhou	Southwest China
156	Weiningmianhual	Weining	Guizhou	Southwest China
157	Weiningmopanli	Weining	Guizhou	Southwest China
158	Weiningmuangli	Weining	Guizhou	Southwest China
159	Weiningpidaili	Weining	Guizhou	Southwest China
160	Weiningqingli	Weining	Guizhou	Southwest China
161	Weiningqingpimopan	Weining	Guizhou	Southwest China
162	Weiningsuanqingli	Weining	Guizhou	Southwest China
163	Weiningtiansuanli	Weining	Guizhou	Southwest China
164	Weiningxiangmianli	Weining	Guizhou	Southwest China
165	Weiningxiaohuangli	Weining	Guizhou	Southwest China
166	Weiningzaibaili	Weining	Guizhou	Southwest China
167	Weiningzaoli	Weining	Guizhou	Southwest China
168	Weiningzishengli	Weining	Guizhou	Southwest China
169	Xiaobaizhaotongli	Weining	Guizhou	Southwest China
170	Yingxueli	Weining	Guizhou	Southwest China
171	Xingyihaizi	Xingyi	Guizhou	Southwest China
172	Zunyixueli	Zunyi	Guizhou	Southwest China
173	Cangxixueli	Cangxi	Sichuan	Southwest China
174	Damali	Cangxi	Sichuan	Southwest China
175	Dahuali	Jianyang	Sichuan	Southwest China
176	Jiangyangdabaili	Jianyang	Sichuan	Southwest China
177	Chenjiadamali	Jinchuan	Sichuan	Southwest China
178	Chengxiangdamali	Jinchuan	Sichuan	Southwest China
179	Hongxiangli	Jinchuan	Sichuan	Southwest China
180	Hanyuanbanjinli	Hanyuan	Sichuan	Southwest China
181	Hanyuanshishengli(1)	Hanyuan	Sichuan	Southwest China
182	Hanyuanzhaobaoli	Hanyuan	Sichuan	Southwest China
183	Jinhuali	Hanyuan	Sichuan	Southwest China
184	Ludingshishengli(2)	Hanyuan	Sichuan	Southwest China
185	Shangengzifengli	Hanyuan	Sichuan	Southwest China
186	Huilihuoba	Huili	Sichuan	Southwest China
187	Huilishibingli	Huili	Sichuan	Southwest China
188	Huilixianhuangli	Huili	Sichuan	Southwest China
189	Huilizaobai	Huili	Sichuan	Southwest China
190	Huilichangbali	Huili	Sichuan	Southwest China
191	Huilixueli	Huili	Sichuan	Southwest China
192	Ludingqingpifengli	Luding	Sichuan	Southwest China
193	1237	Tongliang	Sichuan	Southwest China
194	Baihuali	Tongliang	Sichuan	Southwest China
195	Jindiaozi	Tongliang	Sichuan	Southwest China
196	Jinzhuoguli	Tongliang	Sichuan	Southwest China
197	Ruifu	Tongliang	Sichuan	Southwest China
198	Zhuzuiba	Tongliang	Sichuan	Southwest China
199	Baozhuli	Chenggong	Yunnan	Southwest China
200	Dabaozhu	Chuxiong	Yunnan	Southwest China
201	Huobali	Chuxiong	Yunnan	Southwest China
202	Kunmingmali	Chuxiong	Yunnan	Southwest China
203	Shuaili	Chuxiong	Yunnan	Southwest China
204	Dalihuoba	Dali	Yunnan	Southwest China
205	Dalisuanli	Dali	Yunnan	Southwest China
206	Dalixueli	Dali	Yunnan	Southwest China
207	Fuyuanhuangli	Fuyuan	Yunnan	Southwest China
208	Xibaqingshuili	Kunming	Yunnan	Southwest China
209	Haidongli	Lijiang	Yunnan	Southwest China
210	Lijiangdazhonggu	Lijiang	Yunnan	Southwest China
211	Lijiangdazhonggu	Lijiang	Yunnan	Southwest China
212	Lijianghuangbali	Lijiang	Yunnan	Southwest China
213	Lijianghuangsuanli	Lijiang	Yunnan	Southwest China
214	Lijiangmazhanli(2)	Lijiang	Yunnan	Southwest China
215	Lijiangmanialoli	Lijiang	Yunnan	Southwest China
216	Lijiangmianli	Lijiang	Yunnan	Southwest China

(Continued on next page)

The estimate of Wright's F_{ST} across 10 populations of sand pear landraces was 0.129, suggesting that $\approx 87\%$ of the total genetic variance resided within populations. Furthermore, AMOVA revealed that 88.60% of the total molecular variation was attributed to differentiation among individuals within populations, and only a small amount of variation was partitioned among regions (3.85%) and among populations (7.55%), yet there was a significant level of differentiation among regions and populations ($P < 0.01$) (data now shown).

Genetic relatedness of sand pear landraces from different provinces and regions. Nei et al.'s (1983) genetic distance (D_A) for all possible pairwise population comparisons ranged from 0.450 between Guangdong and Guangxi to 0.792 between Guangdong and Jiangxi (Table 5). The UPGMA based on D_A values revealed three main clusters, which show clear genetic relationships reflecting their geographic distribution (Fig. 2). Each cluster was further subdivided into two subclusters; Cluster I was made up of three populations from east China, including Jiangxi, Zhejiang, and Fujian Provinces. Three populations from southwest China were grouped into Cluster II, whereas Cluster III has two subclusters consisting of two populations (Guangdong, Guangxi) in south and southwest China and two populations (Hubei, Hunan) in central China.

The relationships revealed by principal components analysis (PCA) were similar to those revealed by UPGMA. The first three components of the PCA explained more than 55% of the variation found in the D_A genetic distance matrix. The bidimensional scatterplot strongly differentiated geographically all of the populations except that from Jiangxi (Fig. 3). The first two components, which accounted for 27.62% and 14.39% of total variation, respectively, divided the 10 populations into three major groups and a single population, Jiangxi.

Discussion

Effective conservation of plant germplasm requires a thorough understanding of the existing genetic diversity in the species being studied, its genetic structure and geographic distribution and whether it is to be conserved ex situ or in situ (Allard, 1988; Hamrick and Godt, 1997). Molecular markers, for example, AFLP, RAPD, RFLP, ISSR, and SSR, have been used to determine relationships between a limited number of sand pear cultivars (Cao et al., 2007; Kimura et al., 2002; Shen et al., 2006; Teng et al., 2001, 2002; Zhang et al., 2007) but a genetic analysis of the extant gene pool made up from the large number of traditional landraces in China has not previously been attempted. The present study provides most of the information now available on the extent of genetic diversity within sand pear landraces, information that is required for germplasm repository management in China. As was also

Table 1. (Continued) Chinese landraces of sand pear evaluated.

Accession number	Landrace	Original place of collection		
		District	Province (population)	Region
217	Lijiangpopulu	Lijiang	Yunnan	Southwest China
218	Lijiangtapili	Lijiang	Yunnan	Southwest China
219	Lijiangtianzhonggu	Lijiang	Yunnan	Southwest China
220	Lijiangxiaohuangli	Lijiang	Yunnan	Southwest China
221	Lijiangzhimali	Lijiang	Yunnan	Southwest China
222	Suomeili	Lijiang	Yunnan	Southwest China
223	Sangpili	Lijiang	Yunnan	Southwest China
224	Zhonggushiyueli	Lijiang	Yunnan	Southwest China
225	Miduhuangpili	Midu	Yunnan	Southwest China
226	Midushenwali	Midu	Yunnan	Southwest China
227	Miduxiaomianli	Midu	Yunnan	Southwest China
228	Miduyuli	Midu	Yunnan	Southwest China
229	Hongyunyihao	Unknown	Yunnan	Southwest China
230	Hongyunerhao	Unknown	Yunnan	Southwest China
231	Lushali	Unknown	Yunnan	Southwest China
232	Qiubaili	Unknown	Yunnan	Southwest China
233	Wenshanhongli	Unknown	Yunnan	Southwest China

Fig. 1. Sixty-six geographic origins of 233 sand pear landraces evaluated in this study. 1) Yiwu; 2) Lishui; 3) Yunhe; 4) Leqing; 5) Shouning; 6) Fuan; 7) Pingnan; 8) Pucheng; 9) Jianyang; 10) Jianou; 11) Shunchang; 12) Jinjiang; 13) Jiujiang; 14) Wuyuan; 15) Shangrao; 16) Lianping; 17) Huiyang; 18) Gaoyao; 19) Fengkai; 20) Wuzhou; 21) Guanyang; 22) Gongcheng; 23) Longsheng; 24) Guilin; 25) Lipu; 26) Beiliu; 27) Liucheng; 28) Hengxian; 29) Wuming; 30) Leye; 31) Baise; 32) Tianyang; 33) Debao; 34) Jingxi; 35) Linwu; 36) Yizhang; 37) Longhui; 38) Jingxian; 39) Anjiang; 40) Baojing; 41) Wuhan; 42) Suizhou; 43) Yuanan; 44) Badong; 45) Jianshi; 46) Xuanen; 47) Xianfeng; 48) Lichuan; 49) Meitan; 50) Zunyi; 51) Xingyi; 52) Weining; 53) Tongliang; 54) Cangxi; 55) Jianyan; 56) Jinchuan; 57) Luding; 58) Hanyuan; 59) Huili; 60) Lijing; 61) Dali; 62) Fuyuan; 63) Midu; 64) Kunming; 65) Chuxiong; 66) Chenggong.

found by Kimura et al. (2002), SSRs are particularly useful for assessing levels of genetic variation in cultivated sand pears. All except two of the primer pairs tested allowed reliable scoring of alleles across 233 landraces. The 14 primer pairs used all appear to be hypervariable and generated a total of 184 different alleles, which made it possible to discriminate unequivocally all the sand pear genotypes studied. The average estimates of genetic diversity for each locus ($A = 13.1$, $H_o = 0.671$, $PD = 0.91$) found in this study were slightly different from those ($A = 14.8$, $H_o = 0.63$, $PD = 0.91$) derived by Kimura et al. (2002) in their work on *Pyrus* species. The higher than expected heterozy-

gosity (H_o) observed by us in sand pear landraces indicates that they are genetically more diverse than the 60 pear accessions from six *Pyrus* species studied by Kimura et al. (2002). However, the slightly lower value of A could be the result of different sets of SSR primer pairs being used in the two studies and/or to unique alleles not present in *P. pyrifolia*.

The extent and distribution of genetic diversity within a plant species depends on its evolution and breeding systems, ecological and geographical factors, and human activities. Although sand pears were generally believed to lack morphological variation (Yamamoto et al., 2002a, 2002b), the high

SSR diversity observed in all populations appears to be related to the evolutionary history of the species. First, sand pears were and still are widely distributed throughout south-central China, including at least 13 provinces. Such a large number of sand pear landraces accumulated during the long history of selection and cultivation should have generated tremendous genetic variation in adaptation to different environments. This high genetic diversity would be maintained despite the intense but narrowly focused selection under the complex ecogeographic factors such as latitude, altitude, temperature, and rainfall. Second, the self-incompatibility mating system of *P. pyrifolia* could effectively prevent genetic homogenization within each population as evidenced by the highly discriminatory power of the SSR procedure (Table 3). The rich genetic diversity in sand pears provides an opportunity to broaden the genetic base of modern pear cultivars. Although the Guangxi, Guizhou, Sichuan, and Yunnan populations were slightly more diverse genetically than the other populations, when all estimates of genetic diversity were considered, the difference was not significant except for the Jiangxi population being the least diverse (Table 2). The provincial populations with the highest mean number (8,500) of alleles per locus were those in Guizhou and Guangxi. The regional population with the most alleles per locus (11,428) was from southwestern China; this regional population also showed the highest values for A_e and I (Table 2), suggesting that the Guizhou population should perhaps be a priority for conservation.

It is likely that the genetic diversity of the sand pear gene pool may have been underestimated because that the landraces used in this analysis were only some representatives from different parts of the geographic distribution (Table 1). For example, the 11 landraces in Jiangxi were collected from only three districts with six of them collected from Wuyuan and four and one from Shangrao and Jiujiang, respectively; 87.5% of the landraces (28 of 32 accessions) in Guizhou were derived from a single county, Weining, an area with a long history of pear cultivation (Fig. 1). Therefore, we can reasonably conclude that there are extensive sand pear resources yet to be collected and assessed. Further work should be focused on those districts that have not so far been studied considering the significant genetic differentiation among and between regions. A total of 20 unique alleles was found in 23 landraces belonging to eight different populations. These local alleles could be associated with certain adaptive characteristics, which would be of interest to plant breeders for cultivar improvement and should be given priority for conservation.

At the regional level, southwestern China contains the most diverse sand pear germplasm with slightly lower genetic diversity in central China (Table 2). Our results provide further support for the hypothesis that *P. pyrifolia* originated from southwest China,

Table 2. Estimates of genetic diversity of sand pear in different provinces and regions.

Population	N^z	$A^y \pm SE$	$A_e^x \pm SE$	$H_e^w \pm SE$	$I^v \pm SE^u$
East China	45	9.857 $\pm$ 0.694	5.080 $\pm$ 0.404	0.780 $\pm$ 0.027	1.824 $\pm$ 0.080
Fujian	15	7.500 $\pm$ 0.511	4.764 $\pm$ 0.377	0.773 $\pm$ 0.018	1.716 $\pm$ 0.072
Jiangxi	11	5.643 $\pm$ 0.038	3.149 $\pm$ 0.258	0.655 $\pm$ 0.027	1.322 $\pm$ 0.066
Zhejiang	19	7.429 $\pm$ 0.500	4.587 $\pm$ 0.377	0.747 $\pm$ 0.018	1.641 $\pm$ 0.072
South China	54	8.857 $\pm$ 0.653	5.249 $\pm$ 0.425	0.784 $\pm$ 0.036	1.794 $\pm$ 0.096
Guangdong	13	6.071 $\pm$ 0.530	3.917 $\pm$ 0.380	0.719 $\pm$ 0.021	1.482 $\pm$ 0.085
Guangxi	41	8.500 $\pm$ 0.532	5.071 $\pm$ 0.541	0.773 $\pm$ 0.024	1.765 $\pm$ 0.090
Central China	41	8.785 $\pm$ 0.800	4.115 $\pm$ 0.437	0.731 $\pm$ 0.021	1.617 $\pm$ 0.086
Hubei	26	7.857 $\pm$ 0.762	3.956 $\pm$ 0.511	0.714 $\pm$ 0.023	1.552 $\pm$ 0.088
Hunan	15	6.429 $\pm$ 0.571	3.832 $\pm$ 0.369	0.706 $\pm$ 0.028	1.477 $\pm$ 0.093
Southwest China	93	11.428 $\pm$ 0.850	5.400 $\pm$ 0.507	0.780 $\pm$ 0.033	1.863 $\pm$ 0.099
Guizhou	32	8.500 $\pm$ 0.769	4.953 $\pm$ 0.541	0.758 $\pm$ 0.035	1.711 $\pm$ 0.111
Sichuan	26	8.286 $\pm$ 0.588	4.628 $\pm$ 0.548	0.742 $\pm$ 0.031	1.675 $\pm$ 0.097
Yunnan	35	8.357 $\pm$ 0.427	4.380 $\pm$ 0.360	0.740 $\pm$ 0.033	1.651 $\pm$ 0.082

^zNumber of landraces.^yMean number of alleles per locus (for each region, all the alleles were considered).^xEffective number of alleles per locus.^wNei's expected heterozygosity.^vShannon-Weaver's information index.^uSD.

Table 3. Simple sequence repeat (SSR) primers and estimates of SSR polymorphism parameters based on 233 Chinese sand pear landraces.

Locus code	Size range (bp)	A	H_e	H_o	V	PD
NH001c	118–160	9	0.661	0.500	0.154	0.88
NH002b	148–200	13	0.718	0.733	0.152	0.92
NH004a	78–130	17	0.748	0.844	0.124	0.86
NH005b	304–350	9	0.527	0.554	0.136	0.77
NH007b	120–152	15	0.679	0.738	0.130	0.88
NH008b	188–215	10	0.747	0.669	0.120	0.93
NH009b	138–170	16	0.694	0.518	0.136	0.92
NH011b	151–196	18	0.736	0.695	0.133	0.94
NH013a	190–225	12	0.695	0.451	0.106	0.90
NH014a	60–130	21	0.825	0.802	0.097	0.97
NH015b	100–136	13	0.715	0.740	0.116	0.94
NH017a	88–120	13	0.717	0.731	0.156	0.94
CH01F02 ^z	163–184	12	0.743	0.672	0.106	0.95
CH01H10 ^z	97–123	6	0.666	0.747	0.136	0.90
Mean		13.1	0.705	0.671	0.129	0.91

^zDeveloped from apple (Gianfranceschi et al., 1998).

Table 4. Unique simple sequence repeat alleles in eight pear landrace populations.

Population	Locus	Allele (bp)	Landrace (no.) ^z
Fujian	NH004a	83	Eli (007)
	NH014a	110	Panli (013)
Jiangxi	NH009b	164	Jiangwanxipili (021), Yousu (026)
Zhejiang	NH004a	130	Jiuzhongli (030)
	NH011b	172	Zhenxiangli (033)
Guangxi	NH008b	206	Quanzhouli (077)
	NH009b	148	Cangwudashali (099)
	CH01F02	176	Yanshanhuangpaxiao (078)
	NH004a	98	Weiningzaoli (167)
Guizhou	NH007b	146	Weiningxiaohuangli (165)
	NH009b	138	Yingxueli (170)
	CH01F02	172	Weiningbaipili (144)
	CH01F02	184	Weiningqingpimopan (161)
Hubei	NH002b	148	Make (109), Xianfengbaijie (112)
	NH004a	123	Xianfengbaijie (112)
	NH017a	120	Lichuanxiangshui (104), Wangshuibai (125)
Sichuan	NH002b	160	Huilianghuangli (188)
	NH007b	136	Hanyuanzhaobao (182)
	NH015b	132	Shangengzifengli (185)
Yunnan	NH017a	117	Dalisuanli (205), Miduhuangpili (225)

^zNumbers in parentheses are the accession numbers listed in Table 1.

including Guizhou, Sichuan, and Yunnan Provinces (Rubtsov, 1944). It is interesting to note that the sand pear populations in eastern China (Fujian, Jiangxi, and Zhejiang) are more diverse than those in central China (Hubei and Hunan). This might be the result

of ancient gene flow through cultivar exchanges by local pear growers in different regions of China. Historically, pear production in eastern China was closely associated with Japan and Korea, where sand pears are also distributed, and probably provided addi-

tional opportunities for genetic exchanges with foreign cultivars developed in Japan. RAPD analysis of *Pyrus* species and cultivars revealed close genetic relationships between cultivars from Fujian, Zhejiang, and Japan, indicating ancient gene flow among those regions (Teng et al., 2002).

Analysis of molecular variance showed that most genetic diversity of landraces was within populations with smaller, although still significant, amounts of genetic variation between regions and populations. The estimate of Wright's F_{ST} (0.129) was also higher for sand pears than for wild populations of plant species with similar life histories (F_{ST} = 0.084, Hamrick et al., 1992). This fact reflects the outstanding characteristic of landraces; they are the result of selection through climatic and edaphic adaptation and human activities (Harlan, 1975a, 1975b). Indeed, local crop races are the consequences of long periods of interaction between the environment and genetic systems (Brush, 1995). During the long history of cultivation, farmers have made direct selection for desirable genotypes under the specific agricultural regimes resulting in accumulated genetic differentiation among populations.

The UPGMA dendrograms show that the 10 populations formed region-specific clusters (Fig. 2). Three populations in east China grouped into a distinct subcluster, suggesting profound genetic divergence from other populations, which might be the result of the *P. pyrifolia* distribution. In addition, landraces in this region maybe have an ancient gene introgression with genotypes from Japan and/or Korea, which further increased differentiation with landraces of inland China. Similar region-specific clusters were also evident in the PCA plots, except that the Jiangxi population cannot be grouped with the Fujian and Zhejiang populations (Fig. 3). Furthermore, the Fujian population was, surprisingly, more similar to the Guizhou and Yunnan populations than to its two bordering populations in Jiangxi and Guangdong as shown by the genetic distance matrix (Table 5) and PCA plots (Fig. 3). One possible explanation is that the landraces collected from those populations were from the same latitude and similar climate conditions may force landraces into similar ecotypes. Populations from Hubei and Hunan, Guangdong and Guangxi, and Guizhou, Yunnan, and Sichuan generated similar clusters in the UPGMA dendrograms and PCA plots, which reflected their geographic relationships. The three distinct clusters of the 233 landraces suggest that east China, south China, and southwest China have different germplasm pools and should each be conserved.

In summary, the present study has demonstrated that SSR markers provide an effective tool for assessing genetic diversity and relationships within sand pear germplasm. Further work should focus on combining molecular data and pomological traits for delineating a core collection of sand pear and developing effective conservation strategies and breeding programs.

Table 5. Genetic distance (D_A , Nei et al., 1983) matrix of 10 populations of *Pyrus pyrifolia* in China.

Population	Fujian	Jiangx.	Zhej.	Guangd.	Guangx.	Hube.	Hun.	Guiz.	Sichu.	Yunn.
Fujian	0.000									
Jiangxi	0.659	0.000								
Zhejiang	0.548	0.472	0.000							
Guangdong	0.603	0.792	0.555	0.000						
Guangxi	0.610	0.722	0.529	0.450	0.000					
Hubei	0.641	0.767	0.577	0.598	0.475	0.000				
Hunan	0.645	0.779	0.611	0.582	0.574	0.520	0.000			
Guizhou	0.562	0.744	0.505	0.575	0.544	0.522	0.619	0.000		
Sichuan	0.648	0.733	0.564	0.613	0.584	0.613	0.615	0.595	0.000	
Yunnan	0.535	0.744	0.527	0.541	0.519	0.549	0.567	0.506	0.465	0.000

Fig. 2. Unweighted pair group with arithmetic average dendrograms of 10 populations of sand pear based on Nei et al.'s (1983) genetic distance (D_A).

Fig. 3. Plot of first and second principal components of principal components analysis on 10 populations of sand pear, together accounting for 42.01% of the total variation.

Literature Cited

Allard, R.W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. *J. Hered.* 79:225–238.

Brush, S.B. 1995. *In situ* conservation of landraces in centres of crop diversity. *Crop Sci.* 35:346–354.
Cao, Y.F., F.Z. Liu, Y. Gao, L.J. Jiang, K. Wang, Z.Y. Ma, and K.C. Zhang. 2007. SSR analysis

of genetic diversity of pear cultivars. *Acta Hort. Sinica* 34:305–310.
Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19:11–15.
Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction sites. *Genetics* 131:479–491.
Fan, T.W., D.Y. Cai, H.X. Li, F.L. Wang, C.Z. Zhao, and Y.W. Teng. 2007. Simple sequence repeat (SSR) analysis for assessment of genetic variation and relationships in pear germplasm native to the middle area of Gansu province. *J. Fruit Science* 24:268–275.
Gianfranceschi, L., N. Seglias, and R. Tarchini. 1998. Simple sequence repeats for the genetic analysis of apple. *Theor. Appl. Genet.* 96:1069–1076.
Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53:325–338.
Hamrick, J.L. and M.J.W. Godt. 1997. Allozyme diversity in cultivated crops. *Crop Sci.* 37:26–30.
Hamrick, J.L., M.J.W. Godt, and S.L. Sherman-Broyles. 1992. Factors influencing levels of genetic diversity in woody plant species. *New For.* 6:95–124.
Harlan, J.R. 1975a. Geographic patterns of variability in some cultivated plants. *J. Hered.* 66:184–191.
Harlan, J.R. 1975b. Our vanishing genetic resources. *Science* 188:618–621.
Iketani, H., T. Manabe, N. Matsuta, T. Akiham, and T. Hayashi. 1998. Incongruence between RFLPs of chloroplast DNA and morphological classification in east Asian pear (*Pyrus* spp.). *Gene. Resour. Crop Ev.* 45:533–539.
Kimura, T., H. Iketani, K. Kotobuki, N. Matsutan, Y. Ban, T. Hayashi, and T. Yamamoto. 2003. Genetic characterization of pear varieties revealed by chloroplast DNA sequences. *J. Hort. Sci. Biotechnol.* 78:241–247.
Kimura, T., Z.S. Yong, M. Shoda, K. Kotobuki, N. Matsuta, T. Hayashi, Y. Ban, and T. Yamamoto. 2002. Identification of Asian pear varieties by SSR analysis. *Breed. Sci.* 52:115–121.
Kloosterman, A.D., B. Budowle, and P. Daselaar. 1993. PCR amplification and detection of the human D1S80 VNTR locus amplification conditions, population genetics and application in forensic analysis. *Intl. J. Legal Med.* 105:257–264.
Lee, G.P., C.H. Lee, and C.S. Kim. 2004. Molecular markers derived from RAPD, SCAR, and the conserved 18S rDNA sequences for classification and identification in *Pyrus pyrifolia* and *P. communis*. *Theor. Appl. Genet.* 108:1487–1491.
Morgante, M. and A. Olivieri. 1993. PCR-amplified microsatellites as markers in plant genetics. *Plant J.* 3:175–182.
Nei, M., F. Tajima, and Y. Tateno. 1983. Accuracy of estimated phylogenetic trees from molecular data. *J. Mol. Evol.* 19:153–170.
Peakall, R. and P.E. Smouse. 2001. GenAlEx V5.1: Genetic analysis in Excel. Population genetic software for teaching and research. Australian National University, Canberra, Australia. <<http://www.anu.edu.au/BoZo/GenAlEx/>>.
Rohlf, F.J. 1997. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 2.0. Exeter Software, Setauket, NY.
Rubtsov, G.A. 1944. Geographical distribution of the genus *Pyrus* and trends and factors in its evolution. *Am. Nat.* 78:358–366.
Sanguinetti, C.J., N.E. Dias, and A.J.G. Simpson. 1994. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17:915–919.

- Shen, Y.Y., Y.W. Teng, and K. Tanabe. 2006. RAPD analysis for genetic assessment of some cultivars of *Pyrus pyrifolia* derived from China and Japan. *Acta Hort. Sinica*. 33:621–624.
- Sneath, P.H.A. and R.R. Sokal. 1973. Numerical taxonomy. Freeman, San Francisco, CA.
- Takezaki, N. and M. Nei. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* 144:389–399.
- Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res.* 17:6463–6471.
- Teng, Y.W., K. Tanabe, F. Tamura, and A. Itai. 2001. Genetic relationships of pear cultivars in Xinjiang, China as measured by RAPD markers. *J. Hort. Sci. Biotechnol.* 76:771–779.
- Teng, Y.W., K. Tanabe, F. Tamura, and A. Itai. 2002. Genetic relationships of *Pyrus* species and cultivars native to East Asia revealed by randomly amplified polymorphic DNA markers. *J. Amer. Soc. Hort. Sci.* 127: 262–270.
- Yamamoto, T., T. Kimura, Y. Sawamura, K. Kotobuki, Y. Ban, T. Hayashi, and N. Matsuta. 2001. SSRs isolated from apple can identify polymorphism and genetic diversity in pear. *Theor. Appl. Genet.* 102:865–870.
- Yamamoto, T., T. Kimura, Y. Sawamura, T. Manabe, K. Kotobuki, T. Hayashi, Y. Ban, and N. Matsuta. 2002a. Simple sequence repeats for genetic analysis in pear. *Euphytica* 124:129–137.
- Yamamoto, T., T. Kimura, M. Shoda, Y. Ban, T. Hayashi, and N. Matsuta. 2002b. Development of microsatellite markers in the Japanese pear (*Pyrus pyrifolia* Nakai). *Mol. Ecol. Notes* 2: 14–16.
- Zhang, D., Q. Shu, Y.W. Teng, M.H. Qiu, L. Bao, and H.J. Hu. 2007. Simple sequence repeat analysis on genetic assessment of Chinese red skinned sand pear cultivars. *Acta Hort. Sinica* 34:47–52.