



# DNA Fingerprinting and Genetic Diversity Analysis with Simple Sequence Repeat Markers of 217 Potato Cultivars (*Solanum tuberosum* L.) in China

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## Abstract

Since 1950, more than 620 potato cultivars have been released from Chinese breeding programs, some of which have similar genetic background and phenotype. In this study, 16 parental cultivars widely used in breeding were used to screen 138 simple sequence repeat (SSR) markers. Out of 138 SSR markers, 20 were polymorphic that were used to analyze the genetic diversity of 217 potato cultivars grown in China. In total, 249 alleles were detected with these 20 markers and 244 of them (97.99%) showed polymorphism. The number of alleles ranged from 7 to 22 with an average of 12.45 alleles per primer and polymorphic information content (PIC) ranged from 0.64–0.93 with an average of 0.83. Fragment sizes varied from 80 to 380 bp. Based on PIC values and the clarity of PCR amplification bands, 11 SSR markers were selected and able to differentiate all of the 217 cultivars. The estimated similarity using these polymorphic SSR markers between the cultivars ranged from 0.63 to 0.99, indicating a narrow genetic base. Fingerprinting and genetic diversity analysis in this study provides useful information for the protection of intellectual property, as well as the exploration and utilization of these potato cultivars.

## Resumen

Desde 1950, se han liberado más de 620 variedades de papa de los programas chinos de mejoramiento, algunos de los cuales tienen antecedentes genéticos y fenotipo similares. En este estudio, se usaron 16 variedades parentales utilizadas ampliamente en mejoramiento, para hacer un estudio de 138 marcadores de repeticiones simples de secuencia (SSR). De los 138 marcadores SSR, 20 fueron polimórficos que fueron usados para analizar la diversidad genética de 217 variedades de papa cultivadas en China. En total, se detectaron 249 alelos con estos 20 marcadores y 244 de ellos (97.99%) mostraron polimorfismo. El número de alelos tuvo un rango de 7–22 con un promedio de 12.45 alelos por iniciador, y el contenido de la información polimórfica (PIC) varió de 0.64–0.93 con un promedio de 0.83. Los tamaños de los fragmentos variaron de 80–380 pb. Con base en los valores PIC y la claridad de las bandas de amplificación, se seleccionaron 11 marcadores SSR y fue posible diferenciar a la totalidad de las 217 variedades. La similitud estimada usando estos marcadores polimórficos SSR entre las variedades varió de 0.63 a 0.99, indicando una base genética angosta. Los análisis de huellas y diversidad genética en este estudio proporcionan información útil para la protección de propiedad intelectual, así como para la exploración y utilización de estas variedades de papa.

**Keywords** *Solanum tuberosum* · Polymorphic information content · Microsatellites · Genetic similarity

**Key message** Out of 138 SSR markers tested, 20 were polymorphic and they were used to fingerprint and analyze the genetic diversity of 217 potato cultivars grown in China. A set of 11 SSR markers was sufficient to differentiate all the cultivars. The genetic diversity analysis suggested narrow genetic background of these cultivars.

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## Introduction

Potato (*Solanum tuberosum* L.) is the third most important food crop in the world. It is adapted to a wide range of environmental conditions (Vreugdenhil 2007) and contains high level of nutrients (Gibson and Kurilich 2013). In addition, it also plays an important role in ensuring food security and promoting farmers' income. In 2016, 162 countries and regions planted 19.25 million hectares of potato and the yield reached 376.83 million tons (<http://www.fao.org/faostat/en/#data/QC>).

China is the largest producer of potato in the world with a growing area of 5.63 million hectares and total production of 97.4 million tons in 2016 (Qu 2016). Potato cultivars grown in China have contributed immensely to increased yields and acted as germplasm resources for cultivar improvement. In China, breeding for crop improvement in potato started in 1950 and currently more than 620 potato cultivars have been released. For a long period, confusion has existed in cultivar names because of missing or incorrect records. Sometimes the same cultivar might be known by different names, and sometimes different cultivars have the same name. Therefore, a quick and accurate identification of different cultivars has great importance for seed production, protection of intellectual property, and commercialization of cultivars in China.

Cultivar identification in potato is mainly based on traditional morphological traits, such as tuber morphology (shape, skin and flesh color, distribution and depth of the eyes), leaf type, flower color, and sprout appearance. However, collecting trait data is time consuming, difficult and sometimes strongly affected by environmental factors (Chimote et al. 2004). In addition, the repeated use of selected elite parental clones in breeding further narrows down the genetic diversity among the cultivars. Therefore, it is difficult to distinguish similar cultivars morphologically. Isozyme markers developed in the 1970s were widely used to directly detect the gene products, and have proved to be useful in identification of different potato cultivars (Stegemann and Schnick 1985; Oliver and Martinez-Zapater 1985; Douches and Ludlam 1991). However, isozyme analysis is reported to be affected by plant developmental stage (Hahn and Grifo 1996), and is also limited by the number of loci that can be used (Karaagac et al. 2014).

DNA-based molecular markers can overcome the above mentioned limitations and have been successfully used in potato fingerprinting. Moreover, to increase the efficiency of breeding programs, molecular markers have also been used for the analysis of genetic relationships in various potato cultivars, allowing breeders to establish a broad genetic base for breeding purposes (Bisognin and Douches 2002). Such markers include random amplified polymorphic DNA (RAPD) (Demeke et al. 1993; Ford and Taylor 1997; Chakrabarti et al. 2001), amplified fragment length polymorphisms (AFLPs) (Milbourne et al. 1997), inter-simple sequence repeats (ISSRs) (Prevost and Wilkinson 1999; Bornet et al. 2002), simple sequence repeats (SSRs) (Raker and Spooner 2002), single nucleotide polymorphisms (SNPs) (Bali et al. 2017) and various combinations of above markers (McGregor et al. 2000; Gorji et al. 2011).

SSR markers are highly polymorphic, co-dominant and the primer sequences are generally well conserved within

and between related species (Karaagac et al. 2014). Additional advantages of SSR markers over dominant marker systems are their high heterozygosity and capacity to reflect ploidy status in potato (Ghislain et al. 2004). Many researchers have therefore developed potato SSR markers from genomic libraries and expressed sequence tags (ESTs) databases (Milbourne et al. 1998; Ashkenazi et al. 2001; Ghislain et al. 2004; Feingold et al. 2005; Ghislain et al. 2009). These markers have been extensively used for DNA fingerprinting (Norero et al. 2002; Coombs et al. 2004; Barandalla et al. 2006; Karaagac et al. 2014; Bali et al. 2017), genetic diversity analysis (Chimote et al. 2004; Ispizúa et al. 2007), germplasm migrations (Ríos et al. 2007), and parental analysis (Spanoghe et al. 2015) in potato.

In the present study, SSR markers were used to fingerprint 217 potato cultivars and to analyze the genetic diversity in Chinese germplasm. The data generated will be used for cultivar identification and future germplasm management programs.

## Materials and Methods

### Plant Material

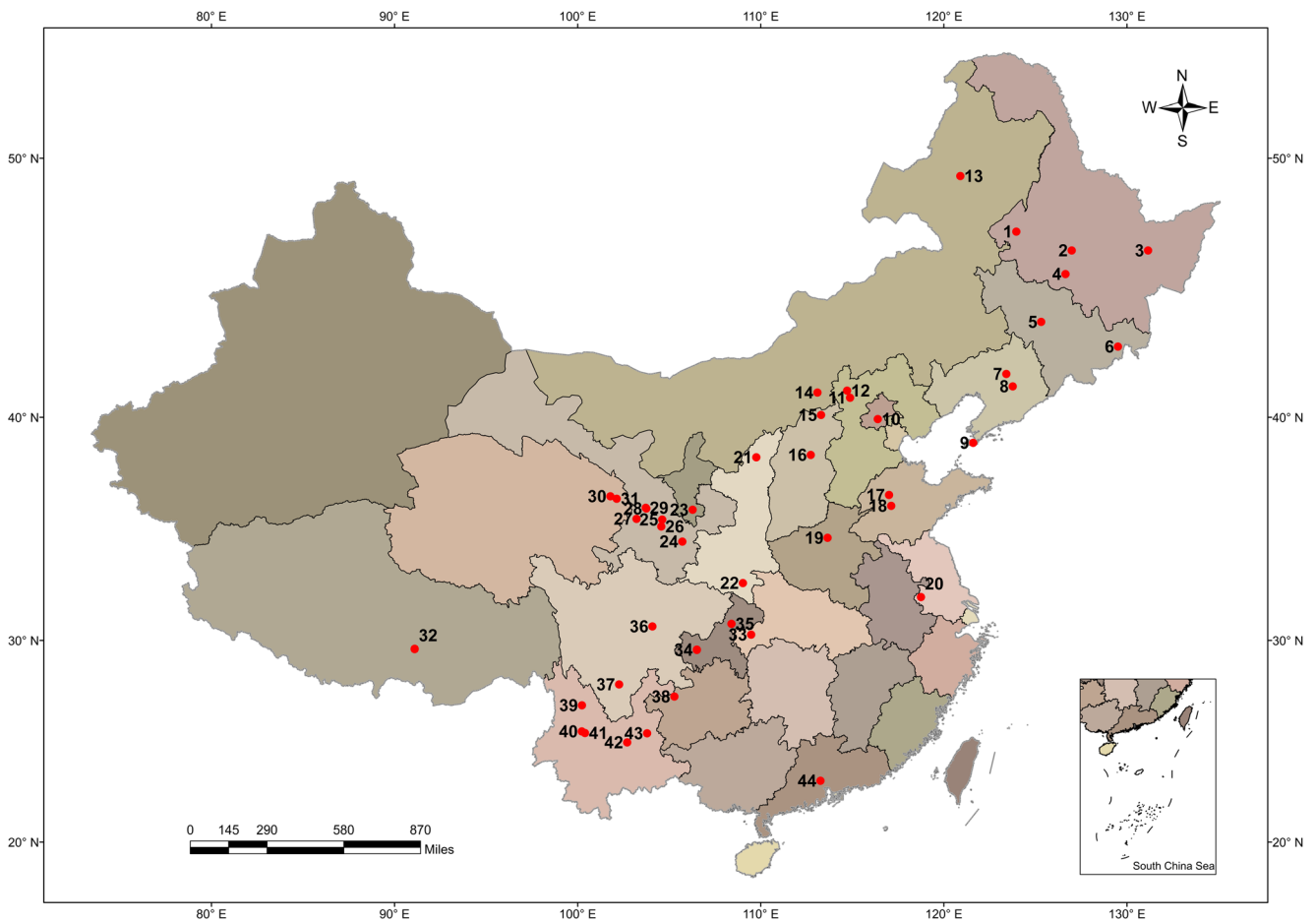
Among the 217 cultivars included in the study, eight (Atlantic, Favorita, Mira, Katahdin, Kuannae, Epoka, Schwalbe, and Anemone) were imported from other countries, and the remaining 209 were bred by 44 regional breeding programs in China from 1950 to 2007 (Fig. 1, Table 1).

Sixteen cultivars that represented the most commonly used parental materials in Chinese potato breeding programs before 2007, were used to screen for polymorphic SSR markers. Katahdin and B76-16 (Xiaoyezi) originated from the United States; Mira, Anemone and Schwalbe originated from Germany; Epoka from Poland; Favorita from the Netherlands; DTO-33 from the International Potato Center (CIP); and, Mengshu 10, Chuanyu 6, Xishu 1, Kexin 2, Gaoyuan 7, Zhongshu 3 and Hutou were domestic cultivars.

### DNA Extraction and SSR Analysis

Genomic DNA was extracted from 2 g of fresh young leaves according to the modified CTAB procedure of Doyle and Doyle (1987) and quantified using 1% agarose gel electrophoresis. The DNA samples were diluted to 25 ng  $\mu\text{L}^{-1}$  and stored at  $-20\text{ }^{\circ}\text{C}$  until use.

A total of 138 markers located on all 12 potato chromosomes were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The primer sequences of these markers were obtained from previous studies (Ghislain et al. 2004;



**Fig. 1** Map of China showing the geographical distribution of the 44 breeding programs of the cultivars used in the present study. Numbers are cited as map locality in Table 1

Feingold et al. 2005). Marker polymorphism was evaluated using the 16 cultivars mentioned above. Polymorphic markers were used to fingerprint and analyze the genetic diversity of 217 potato cultivars.

PCR amplifications were performed in a 20  $\mu\text{L}$  reaction mixture, containing 2.0  $\mu\text{L}$  of 25 ng  $\mu\text{L}^{-1}$  DNA template, 0.8  $\mu\text{L}$  of 10 pmol  $\mu\text{L}^{-1}$  forward primer, 0.8  $\mu\text{L}$  of 10 pmol  $\mu\text{L}^{-1}$  reverse primer, 1.6  $\mu\text{L}$  of 2.5 mmol  $\text{L}^{-1}$  dNTPs, 2.0  $\mu\text{L}$  of 10  $\times$  PCR buffer, 0.4  $\mu\text{L}$  of Taq DNA polymerase (2.5 U  $\mu\text{L}^{-1}$ ) (Tiangen Biotech Co., Ltd., Beijing, China), and 12.4  $\mu\text{L}$  of ddH<sub>2</sub>O. A modified PCR program was used: 5 min at 94  $^{\circ}\text{C}$ , 35 cycles of 30 s at 94  $^{\circ}\text{C}$ , 30 s at SSR specific annealing temperature (53  $^{\circ}\text{C}$  to 64  $^{\circ}\text{C}$ ) and 45 s at 72  $^{\circ}\text{C}$ ; with a final extension step of 7 min at 72  $^{\circ}\text{C}$ . The PCR products were fractionated on 8% denatured polyacrylamide gel electrophoresis, and stained with silver nitrate.

## Data Analysis

A data file was constructed by scoring 0 for absence and 1 for presence of specific amplification product for each sample.

The following related genetic parameters were then calculated:

- (1). Percentage of polymorphic loci  $p = (k/n) \times 100\%$ , where  $k$  is the number of polymorphic loci, and  $n$  is the total number of measured loci.
- (2). Polymorphic information content (PIC)  $= 1 - \sum_1^i f_i^2$ , where:  $f_i$  is the frequency of the  $i^{\text{th}}$  allele in a locus.

Cluster analysis was performed with NTSYS Pc2.11 using the Dice co-efficient (Dice 1945) for calculating similarities and unweighted pair-group arithmetic average method (UPGMA) for constructing dendrograms.

## Results

### SSR Marker Screening

Among the 138 SSR markers screened on 16 potato cultivars (data not shown), 20 SSR markers generated polymorphism. These 20 markers were used to fingerprint 217 cultivars: a

**Table 1** Cultivars analyzed, parentage, breeding programs and map locations

Code	Cultivar	Parentage	Breeding program	Map locality
1	Changshu 3	Unknown	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
2	Changshu 4	Self inbred progeny of Epoka	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
3	Changshu 5	Unknown	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
4	Kexin 1	374–128 × Epoka	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
5	Kexin 2	Mira×Epoka	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
6	Kexin 3	Mira×Katahdin	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
7	Kexin 4	Anemome×Katahdin	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
8	Kexin 5	Anemome×Katahdin	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
9	Kexin 6	S41956 × 96–56	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
10	Kexin 8	Kexin 4 × Kexin 6	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
11	Kexin 9	(Anemome×Zaopuli) × Duozibai	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
12	Kexin 11	CIP7176 × Epoka	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
13	Kexin 12	Self inbred progeny of Dorita	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
14	Kexin 13	Two generation self inbred progeny of Mira	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
15	Kexin 14	S16–1–1-14-1-3-6(5) × A-11–1-8(9)	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
16	Kexin 15	Belmont×Hu 8342–36	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
17	Kexin 16	Beifanghong×KeBP9601	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
18	Kexin 17	F81109 × B5141–6	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
19	Kexin 18	Epoka×374–128	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
20	Kexin 19	Kexin 2 × KPS92–1	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
21	Kexin 20	Fortune×Kexin 2	Potato Research Institute, Heilongjiang Academy of Agricultural Sciences	1
22	Huangmazi	Unknown	Local Cultivar in Wangkui County, Heilongjiang Province	2
23	Jinong 958	Unknown	Jixian Farm, Heilongjiang Province	3
24	Dongnong 303	Anemome×Katahdin	Northeast Agricultural University	4
25	Dongnong 304	S4–5–3-9-1-25-(6) × NS12–156(1)	Northeast Agricultural University	4
26	Dongnong 305	Atlantic×NS12–156–1-1	Northeast Agricultural University	4
27	Dongnong 306	All Blue	Northeast Agricultural University	4
28	Dongnong 307	DDGS-1	Northeast Agricultural University	4
29	Chunshu 1	Red Warba×Katahdin	Institute of Vegetables and Flowers, Jilin Province	5
30	Chunshu 2	Gaoyuan7 × Katahdin	Institute of Vegetables and Flowers, Jilin Province	5
31	Chunshu 3	S.demissumA6 × Kexin 3	Institute of Vegetables and Flowers, Jilin Province	5
32	Chunshu 4	Wensheng 4 × Kexin 2	Institute of Vegetables and Flowers, Jilin Province	5
33	Chunshu 5	Chunshu 2 × Ke S2–14–1-12-3(9)	Institute of Vegetables and Flowers, Jilin Province	5
34	Tuqiang 2	Zaopuli×Heilongjiang3	Institute of Vegetables and Flowers, Jilin Province	5
35	Yanshu 4	Liesiji from Moscow	Yanbian Korean Autonomous Prefecture Academy of Agricultural Sciences, Jilin Province	6
36	Liaoling 1	5903–2 × Epoka	Institute of Crop Sciences, Liaoning Academy of Agricultural Sciences	7
37	Jingkengbai	Epoka×Duozibai	Benxi Institute of Agricultural Sciences, Liaoning Province	8
38	Youjin	NS80–31 × 8023–10	Benxi Institute of Agricultural Sciences, Liaoning Province	8
39	Zaodabai	Wulibai×74–128	Benxi Institute of Agricultural Sciences, Liaoning Province	8
40	Chaobai	372–18 × Kexin 3	Dalian Institute of Agricultural Sciences, Liaoning Province	9
41	Zhongda 1	w <sub>2</sub> /D-6-1	Institute of Vegetables and Flowers of Chinese Academy of Agricultural Sciences, Daxinganling Institute of Agricultural Sciences	10
42	Zhongshu 1	Dongnong 303 × NS79–12-1	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
43	Zhongshu 2	LT-2 × DTO-33	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
44	Zhongshu 3	Jingfeng 1 × BF67A	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
45	Zhongshu 4	Dongnong 3012 × 85 T-13-8	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
46	Zhongshu 5	Self inbred progeny of ZhongShu 3	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
47	Zhongshu 6	85 T-13-8 × NS79–12-1	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
48	Zhongshu 7	Zhongshu 2 × Jizhangshu 4	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
49	Zhongshu 8	W953 × FL475	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
50	Zhongshu 9	Shepody×Zhongshu 3	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
51	Zhongshu 10	F79055 × ND860–2	Institute of Vegetables and Flowers of Chinese Academy of Agricultural Sciences, Potato Research Centre, Agriculture and Agri-Food Canada	10
52	Zhongshu 11	Aminca×Chaleur	Institute of Vegetables and Flowers of Chinese Academy of Agricultural Sciences, Potato Research Centre, Agriculture and Agri-Food Canada	10
53	Zhongshu 12	W953 × FL475	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
54	Zhongshu 13	Shepody×Zhongshu 3	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
55	Zhongshu 14	Shepody×Zhongshu 3	Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences	10
56	Zhongxin 24	Unknown	Chinese Academy of Agricultural Sciences	10
57	Bashu 5	Amuxier×Fushanhongyanquan	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
58	Bashu 7	35–131 × 73–21–1	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
59	Bashu 9	Duozibai×Epoka	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
60	Bashu 10	Hutou×Schwalbe	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
61	Hutou	Zishanyao×B76–16 (Xiaoyezi)	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
62	Jizhangshu 3	Ostara	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
63	Kangbingchi	Zishanyao×Epoka	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11

**Table 1** (continued)

Code	Cultivar	Parentage	Breeding program	Map locality
64	Shaza 1	Nanjue×Epoka	Zhangjiakou Bashang Institute of Agricultural Sciences, Hebei Province	11
65	Jizhangshu 8	720,087 × X4.4	High Latitude Crops Institute, Hebei Provinces	12
66	Zhangshu 7	Yagana×XY.20	High Latitude Crops Institute, Hebei Provinces	12
67	Hushu 1	Kexin 2 × (Fengshoubai×Duozibai)	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
68	Hushu 4	Anemome×Changshu 4	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
69	Hushu 5	Kexin 2 × Duozibai	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
70	Hushu 8	Hu 8209 × Hudan 81–118	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
71	Mengshu 9	543 × Hudan 81–149	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
72	Mengshu 10	Hudan 81–118 × Hudan 80–298	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
73	Mengshu 12	546 × Hudan 81–149	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
74	Mengshu 13	Hongwenbai×Hudan 81–149	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
75	Neishu 7	Hudan 80–298 × Hu 8206	Hulun Buir Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	13
76	Mengshu 11	Unknown	Ulanqab Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	14
77	Wumeng 601	B76–16 (Xiaoyezi) × Duozibai	Ulanqab Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	14
78	Wumeng 684	Fuluduo×Duozibai	Ulanqab Institute of Agricultural Sciences, Inner Mongolia Autonomous Region	14
79	Jinshu 1	Arran Peak×Argo	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
80	Jinshu 2	Ebro×Industria	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
81	Jinshu 3	Heishanya×Kennibaik	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
82	Jinshu 4	Liwaihaung×Duozibai	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
83	Jinshu 5	Jinshu 2 × Schwalbe	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
84	Jinshu 6	Jinshu 2 × Schwalbe	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
85	Jinshu 7	6401–3–35 × Schwalbe	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
86	Jinshu 8	Jinshu 2 × NS78–7	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
87	Jinshu 11	H319–1 × NT/TBULK	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
88	Jinshu 13	K299 × Jinshu 7	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
89	Jinshu 14	9201–59 × (6401–3–35 × Schwalbe)	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
90	Jinshu 15	Jin 11 × 9424–2	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
91	Jinshu 16	NL94014 × 9333–11	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
92	Jinshu 17	II-14[8408–22 × (Jinshu 6 × Solanum chacoense)]/NS78–7	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
93	Tongshu 5	Zishanya×Epoka	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
94	Tongshu 9	Liwaihaung×Argo	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
95	Tongshu 20	(8408–22//Jinshu6/S.chacoense)/NS78–7	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
96	Tongshu 22	Jinshu 11 × Jinshu 7	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
97	Tongshu 23	8029-[S2–26–13-(3)]/NS78–4//Helan 7	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
98	Xishu 1	Self inbred progeny of Duozibai	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
99	Yanping 1	Lanyan×Katahdin	High Latitude Crops Institute, Shanxi Academy of Agricultural Sciences	15
100	Jinshu 9	Shengli 2 × Schwalbe	Wuzhai Experimental Station, Shanxi Academy of Agricultural Sciences	16
101	Jinshu 10	Self inbred progeny of 81–5-6	Wuzhai Experimental Station, Shanxi Academy of Agricultural Sciences	16
102	Jinshu 12	75–30-7 × Schwalbe	Wuzhai Experimental Station, Shanxi Academy of Agricultural Sciences	16
103	Lupotato 3	BL61–74-167 × And77–1347-280	Institute of Vegetables, Shandong Academy of Agricultural Sciences	17
104	Shuangfeng 4	Co-rina×Fengshoubai	Institute of Vegetables, Shandong Academy of Agricultural Sciences	17
105	Shuangfeng 5	IROSE×Fengshoubai	Institute of Vegetables, Shandong Academy of Agricultural Sciences	17
106	Shuangfeng 6	83,119-(10) × PVY-31	Institute of Vegetables, Shandong Academy of Agricultural Sciences	17
107	Taishan 1	Anemome×(Anemome×Katahdin)	Shandong Agricultural University	18
108	Zhengshu 2	Anemome×Kexin 2	Zhengzhou Institute of Vegetables, Henan Province. Potato Research Institute of Heilongjiang Academy of Agricultural Sciences	19
109	Zhengshu 5	Gaoyuan 7 × Zheng 762–93	Zhengzhou Institute of Vegetables, Henan Province	19
110	Zhengshu 6	Gaoyuan 7 × Zheng 762–93	Zhengzhou Institute of Vegetables, Henan Province	19
111	Zhengshu 7	Favorita×Zhengshu 5	Zhengzhou Institute of Vegetables, Henan Province	19
112	B76–16 (Xiaoyezi)	96–44 × 528–170	Former Central Agricultural Institute	20
113	Fengshou	Huoma×B76–23	Former Central Agricultural Institute	20
114	Wuxia	B76–43(96–44 × 528–170)	Former Central Agricultural Institute	20
115	Shaza 15	Jinpingguo×Duozibai	Yulin Agricultural Research Institute, Shaanxi Provinces	21
116	Annong 5	Self inbred progeny of Hajiao 25	Ankang Institute of Agricultural Sciences, Shaanxi Provinces	22
117	Anshu 56	175 × Kexin 2	Ankang Institute of Agricultural Sciences, Shaanxi Provinces	22
118	Qinyu 30	Epoka×4081	Ankang Institute of Agricultural Sciences, Shaanxi Provinces	22
119	Qinyu 31	Yun 94–51 × 89–1	Ankang Institute of Agricultural Sciences, Shaanxi Provinces	22
120	Wensheng 4	Self inbred progeny of Changshu 4	Ankang Institute of Agricultural Sciences, Shaanxi Provinces	22
121	Ningshu 1	Gan 65–17-1 × Gan 65–15-7	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
122	Ningshu 4	Self inbred progeny of Lanhuayangyu	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
123	Ningshu 5	Self inbred progeny of 76–2-15	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
124	Ningshu 6	Self inbred progeny of BESON	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
125	Ningshu 7	Ningshu 1 × (Aputa×71–18-2)	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
126	Ningshu 8	Self inbred progeny of Shenyawo	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
127	Ningshu 9	Self inbred progeny of 93	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
128	Ningshu 10	Self inbred progeny of Dongnong 303	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23
129	Ningshu 11	Self inbred progeny of Longshu 3	Guyuan Institute of Agricultural Sciences, Ningxia Hui Autonomous Region	23

**Table 1** (continued)

Code	Cultivar	Parentage	Breeding program	Map locality
130	Tianshu 5	Unknown	Tianshui Institute of Agricultural Sciences, Gansu Province	24
131	Tianshu 7	Tianshu 6 × Weihui 2	Tianshui Institute of Agricultural Sciences, Gansu Province	24
132	Tianshu 8	62–118 × DTO-33	Tianshui Institute of Agricultural Sciences, Gansu Province	24
133	Tianshu 9	91–26–116 × 85–6–14	Tianshui Institute of Agricultural Sciences, Gansu Province	24
134	Xindaping	Unknown	Anding Agricultural Technology Center in Dingxi, Gansu Province	25
135	Weishu 1	Weihui4 × Weihui 2	Huichuan Farm inWeiyuan County, Gansu Province	26
136	Weishu 8	Unknown	Huichuan Farm inWeiyuan County, Gansu Province	26
137	Linshu 2	B76–16 (Xiaoyezi) × Ewerest	Linxia Institute of Agricultural Sciences, Gansu Province	27
138	Linshu 3	B76–16 (Xiaoyezi) × Ewerest	Linxia Institute of Agricultural Sciences, Gansu Province	27
139	Linshu 7	Dahongyanwo × Duoizibai	Linxia Institute of Agricultural Sciences, Gansu Province	27
140	Gannongshu 2	83–1 × P30–1	Gansu Agricultural University	28
141	Gannongshu 3	Unknown	Gansu Agricultural University	28
142	Kangyi 1	Epoka × 3NKNHreH	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
143	Longshu 1	Cornelia × Changshu 4	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
144	Longshu 3	35–131 × 73–21–1	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
145	Longshu 4	62–47/119-II	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
146	Longshu 5	Xiaobaihua × 119–8	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
147	Longshu 6	Wushu 86–6–14 × Longshu 4	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
148	Shengli 1	63–8–27 × 62–1–10	Institute of Food Crops, Gansu Academy of Agricultural Sciences	29
149	Gaoyuan 1	Niutou × Duoizibai	Qinghai Academy of Agricultural and Forestry Sciences	30
150	Gaoyuan 2	Duoizibai × Mira	Qinghai Academy of Agricultural and Forestry Sciences	30
151	Gaoyuan 4	Duoizibai × Mira	Qinghai Academy of Agricultural and Forestry Sciences	30
152	Gaoyuan 5	Shenyanwo × 742	Qinghai Academy of Agricultural and Forestry Sciences	30
153	Gaoyuan 6	Niutou × Deyou 4	Qinghai Academy of Agricultural and Forestry Sciences	30
154	Gaoyuan 7	Gaoyuan 4 × Gaoyuan 3	Qinghai Academy of Agricultural and Forestry Sciences	30
155	Qingshu 2	Gaoyuan 4 × magura	Qinghai Academy of Agricultural and Forestry Sciences	30
156	Qingshu 3	Shenyanwo × Gaoyuan 3	Qinghai Academy of Agricultural and Forestry Sciences	30
157	Qingshu 4	Niutou × Desiree	Qinghai Academy of Agricultural and Forestry Sciences	30
158	Qingshu 5	93–5–1 × 92–32–42	Qinghai Academy of Agricultural and Forestry Sciences	30
159	Qingshu 6	Gu33–1 × 92–9–44	Qinghai Academy of Agricultural and Forestry Sciences	30
160	Qingshu 7	92–32–42 × 92–5–2	Qinghai Academy of Agricultural and Forestry Sciences	30
161	Qingshu 8	Qingshu 2 × Tuodu 175	Qinghai Academy of Agricultural and Forestry Sciences	30
162	Qingshu 168	Fushen 6–3 × Desiree	Qinghai Academy of Agricultural and Forestry Sciences	30
163	Xiazhai 65	Gaoyuan 2 × Star	Huzhu Institute of Agricultural Sciences, Qinghai Province.	31
164	Zangshu 1	Self inbred progeny of Bolan 2	Tibet Institute of Agricultural Sciences	32
165	Epotato 1	674–5 × CFK-69.1	Enshi Southern China Potato Research Center, Hubei Province	33
166	Epotato 3	NS7914.33 × E59–5–86	Enshi Southern China Potato Research Center, Hubei Province	33
167	Epotato 4	Ke 6717–36 × Epotato 1	Enshi Southern China Potato Research Center, Hubei Province	33
168	Epotato 5	393,143–12 × NS51–5	Enshi Southern China Potato Research Center, Hubei Province	33
169	Nanzhong 552	Capella × 78–7	Enshi Southern China Potato Research Center, Hubei Province	33
170	Xinyu 3	Epoka × Mira	Enshi Southern China Potato Research Center, Hubei Province	33
171	Xinyu 4	Aquila × Epoka	Enshi Southern China Potato Research Center, Hubei Province	33
172	Wannong 4	pontiac × Duoizibai	Wanxian Institute of Agricultural Sciences, Sichuan Province	34
173	Wanshu 8	66,116 × Duoizibai	Wanxian Institute of Agricultural Sciences, Sichuan Province	34
174	Wanyu 9	Wuxi × Duoizibai	Wanxian Institute of Agricultural Sciences, Sichuan Province	34
175	Yupotato 1	8911–3(119–3 × Desiree)	Sanxia Institute of Agricultural Sciences, Chongqing	35
176	Chuanyu 4	C1an-dia × 7XY-1	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
177	Chuanyu 5	LT-1 × 377,970.3	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
178	Chuanyu 6	44–4 × Liangshu 3	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
179	Chuanyu 8	Unknown	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
180	Chuanyu 10	44–4 × Liangshu 3	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
181	Chuanyu 39	379,645.4 × 7XY-1	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
182	Chuanyu 56	36–150 × Schwalbe	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
183	Chuanyuzao	7032–2–1 × Schwalbe	Crop Research Institute, Sichuan Academy of Agricultural Sciences	36
184	Liangshu 3	Mira × 9–49	Xichang Institute of Agricultural Sciences, Sichuan Province	37
185	Liangshu 8	Liangshu 97 × A17	Xichang Institute of Agricultural Sciences, Sichuan Province	37
186	Liangshu 14	Self inbred progeny of Kuannae	Xichang Institute of Agricultural Sciences, Sichuan Province	37
187	Liangshu 17	105–16 × Schwalbe	Xichang Institute of Agricultural Sciences, Sichuan Province	37
188	Liangshu 97	6–36 × Schwalbe	Xichang Institute of Agricultural Sciences, Sichuan Province	37
189	Weiyu 3	Self inbred progeny of Kuannae	Weining Institute of Agricultural Sciences, Guizhou Province	38
190	Lishu 1	Self inbred progeny of Kuannae	Lijiang Institute of Agricultural Sciences, Yunnan Province	39
191	Lishu 2	Huzi 79–172 × NS79–12-1	Lijiang Institute of Agricultural Sciences, Yunnan Province	39
192	YushuCA	377,427.1 × 7xy.1	Dali Seed Company, Yunnan Province	40
193	Zhongdianhong	Unknown	Dali Institute of Agricultural Sciences and Diqing Seed Company, Yunnan Province	41
194	Yunshu 101	S95–105 × Neishu 7	Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences	42
195	Yunshu 102	S95–105 × Neishu 7	Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences	42
196	Yunshu 201	S95–105 × Neishu 7	Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences	42
197	Yunshu 301	93–92 × C89–94	Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences	42

**Table 1** (continued)

Code	Cultivar	Parentage	Breeding program	Map locality
198	Yunshu 501	Xuan 92–816 × Xuan 94–232	Industrial Crops Research Institute, Yunnan Academy of Agricultural Sciences	42
199	Cooperation 001	True seed from CIP	Huize Agricultural Technology Center and the Root and Tuber Crops Institute of Yunnan Normal University	43
200	Cooperation 002	True seed from CIP	Huize Agricultural Technology Center and the Root and Tuber Crops Institute of Yunnan Normal University	43
201	Cooperation 003	True seed from CIP	Huize Agricultural Technology Center and the Root and Tuber Crops Institute of Yunnan Normal University	43
202	Cooperation 23	True seed from CIP	Huize Agricultural Technology Center and the Root and Tuber Crops Institute of Yunnan Normal University	43
203	Cooperation 88	“I-1085” × BLK2	Huize Agricultural Technology Center and the Root and Tuber Crops Institute of Yunnan Normal University	43
204	Hui-2	Yinxike×Weihui 2	Huize Agricultural Technology Center, Yunnan Province	43
205	Jinguan	Sprout mutation of Favorita	South China Agricultural University	44
206	Hua 525	Unknown	Unknown	
207	Weibian 94–18	Unknown	Unknown	
208	Wenchuan 9–1	Unknown	Unknown	
209	Zhuanxinwu	Unknown	Local Cultivar in Yunnan Province	
210	Epoka	Stamm913 × Delfin	Imported from Poland	
211	Anemone	Viola×Flava	Imported from Germany	
212	Mira	Capella×B.R.A.089	Imported from Germany	
213	Schwalbe	Aquila×Capella	Imported from Germany	
214	Favorita	ZPC50–35 × ZPC55–37	Imported from the Netherlands	
215	Kuannae	Unknown	Imported from Czech Republic	
216	Atlantic	B5141–6 × Wauseon	Imported from the United States	
217	Katahdin	USDA24642 × USDA40568	Imported from the United States	

total of 249 alleles were obtained, of which 244 alleles were polymorphic (97.99%). The number of alleles in the 20 loci ranged from seven (primers S7 and S122) to 22 (primer S189) with an average of 12.45. The PIC values for the markers varied from 0.64 (primer S122) to 0.93 (primer S189) with an average of 0.83. The size of amplifications ranged from 80 to 380 bp (Table 2).

### Construction of DNA Fingerprinting

The 20 polymorphic SSR markers were used to fingerprint 217 cultivars. Eleven markers (S122, S168, S151, S184, S7, S170, S118, S192, S180, S174, and S25) demonstrated high PIC values, high quality amplifications, and were able to differentiate all 217 cultivars (Supplemental Material 1). These markers produced 132 alleles, of which 129 (97.73%) were polymorphic (Table 2). The DNA fingerprint for each cultivar was unique. Figure 2 shows the amplification of 17 potato cultivars by marker S122. The smallest allele amplified by S122 was designated as 1 and the largest allele amplified was designated as 7.

Thirty-one cultivars produced unique alleles with certain markers (Table 3). For Cooperation 001 and Qinyu 31, three markers generated unique alleles. Kexin 20, Cooperation 002, Cooperation 003, Cooperation 88, Jinshu 14 and Zhongdianhong amplified unique alleles with two markers. Only one marker in the remaining 23 cultivars produced unique alleles.

### Genetic Diversity Analysis

Based on the results from 20 polymorphic SSR markers, a similarity matrix was used to generate a UPGMA dendrogram (Supplemental Material 2). The dendrogram showed that all of the 217 cultivars were closely related and lacked the formation of distinct clusters.

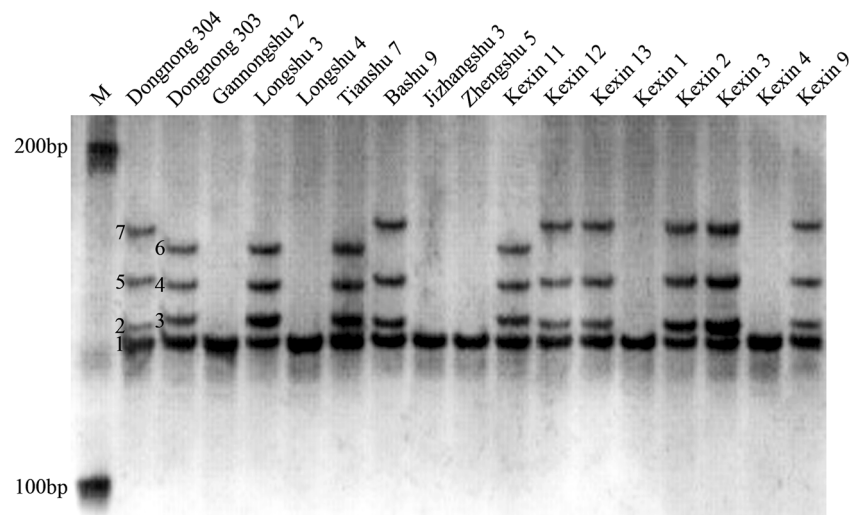
In most cases, the dendrogram grouping fits well with the recorded pedigree information. Cultivars from the same cross usually clustered together, for example, Kexin 4 and Kexin 5 (Anemone X Katahdin); Zhengshu 5 and Zhengshu 6 (Gaoyuan 7 X Zheng 762-93); Zhongshu 8 and Zhongshu 12 (W953 X FL475); and, Yunshu 101, Yunshu 102 and Yunshu 201 (S95-105 X Neishu 7). Cultivars sharing one of the parents also clustered together, such as Kangyi 1, Kangbingchi and Jinkengbai which had Epoka as one of their parents; Wannong 4, Wanshu 8 and Wanyu 9 which had Duoizibai as the male parent; and, Chuanyu 39 and Chuanyu 4 which had 7XY.1 as the male parent. Cultivars usually clustered together with their parent(s). As examples, Jinshu 5 and Jinshu 8 clustered with their female parent, Junshu 2; and, Liangshu 8 clustered together with its female parent, Liangshu 97. Most of these 217 cultivars were progeny of cultivars or accessions from the United States, Germany, Poland, the Netherlands, and CIP, and they were distributed in almost all the clusters. There was no obvious clustering trend by their country of origin: perhaps because a few foreign elite lines were used widely as parental clones in most breeding programs.

**Table 2** Summary of 20 SSR markers used in the present study

Primer code	Chromosome location	Repeat	Primer sequences (5'–3') forward–reverse	Reference	Annealing temperature(°C)	No. of alleles	No. of polymorphic alleles	Ratio of polymorphic alleles (%)	Allele range (bp)	PIC
S122	IX	(ATT) <sub>5</sub>	GACAGTTCACCATAAAA AGAAATAAGCAAAAGCAA	Ghislain et al. 2004	53	7	6	85.71	155–185	0.6407
S168	VII	(CTCC) <sub>n</sub>	CTGCCGCAAAAAGTGAAAAC TGAATGTAGCCCAAATTTGAA	Feingold et al. 2005	60	12	12	100	110–165	0.6639
S151	VI	(AAG) <sub>n</sub>	GCTGCTAAAGACTCAAGCAGAA CAACTACAAGATCCATCCACAG	Feingold et al. 2005	55	12	12	100	80–153	0.8344
S184	II	(CTT) <sub>n</sub>	TCATCACAACTGACCCCA GGGCTTGAATGTGAAGCTC	Feingold et al. 2005	55	17	17	100	160–290	0.8767
S7	II	(CA) <sub>imp</sub> (TC) <sub>imp</sub>	GACTGGCTGACCTGAACCTC GACAAAATTACAGGAACCTGCAAA	Feingold et al. 2005	55	7	7	100	135–160	0.8116
S170	VII	(GAA) <sub>n</sub>	CGCAATCTCATCCGATC TCCGGCGGATAAATCTTGT	Feingold et al. 2005	58	15	15	100	116–270	0.8623
S118	XII	Compound(GT/GC)(GT) <sub>8</sub>	AGAGATCGATGTAAAACACGTT GTGGCAITTTGATGGATT	Ghislain et al. 2004	53	11	11	100	115–190	0.8703
S192	III	(ATA) <sub>n</sub>	ACTTCGCACTGGTGAAGC GGTCTGGATTCCCAGGTTG	Feingold et al. 2005	55	9	8	88.89	160–250	0.7605
S180	VII	(TAA) <sub>n</sub>	ACTGCTGTGGTTGGCGTC ACGGCATAGATTTGGAAGCATC	Feingold et al. 2005	55	15	15	100	130–200	0.9081
S174	VII	(AGG) <sub>n</sub>	TGAGGGTTTTCAGAAAAGGA CATCTTTGCAACAACCTCTCT	Feingold et al. 2005	64	14	14	100	120–195	0.8844
S25	X	(GGC) <sub>n</sub> (GGT) <sub>n</sub>	GCGAATGACAGGACAAAGAGG TGCCACTGCTACCATAACCA	Feingold et al. 2005	55	13	12	92.31	180–380	0.8297
S189	IX	(AAG) <sub>n</sub>	CCTTGTAACAAGCAGTGGTC TCCGCCAAGACTGATGCA	Feingold et al. 2005	55	22	22	100	180–380	0.9324
S182	VI	(TCTT) <sub>n</sub>	GGAACTCCTCAACTGGCTG TCAACTATATGCCCTACTGCCCAA	Feingold et al. 2005	55	16	16	100	146–270	0.8885
S120	II	(TA) <sub>12</sub> ...(TG) <sub>4</sub> GT (TG) <sub>5</sub>	GTCTTTTGGTGGTTTTCTT TTAATTCCTGTGTGGCTG	Ghislain et al. 2004	55	9	8	88.89	180–260	0.8130
S187	IV	(AAG) <sub>n</sub>	CCGTTGATGGGATTCACA TGATATTACCATGGCAGCAGC	Feingold et al. 2005	55	10	10	100	220–380	0.8021
S183	III	(ATA) <sub>n</sub>	TTCTCTAAAGCGCAAAAAG GGAGAGACTTGGGTTTCTCC	Feingold et al. 2005	55	9	9	100	160–210	0.7833
S162	XI	(CAT) <sub>n</sub> (TAG) <sub>n</sub> (AAG) <sub>n</sub>	TATGGAAATTCGGTGTAGG GACGGTGACAAAAGAGAAAGG	Feingold et al. 2005	64	13	13	100	165–265	0.8839
S153	XII	(GTT) <sub>n</sub> (GAT) <sub>n</sub>	TATGTTCCACGCCATTCAG ACGGAAAATCATCTGTCAAT	Feingold et al. 2005	55	18	18	100	125–190	0.9252
S148	IV	(AAT) <sub>n</sub>	CAGCAAAATCAGAACCCGAT GGATCATCAAATTCACCGCT	Feingold et al. 2005	55	12	12	100	180–295	0.8757
S188	II	(TA) <sub>n</sub>	GACAGAGAATATGGGACCACCA GCAGCACCTTAAATGGCTGAC	Feingold et al. 2005	55	8	7	87.5	180–235	0.7706
Total					–	249	244	–	–	–
Average					–	12.45	12.2	97.99	–	0.8309



**Fig. 2** DNA fragments amplified by SSR marker S122 in 17 potato cultivars



Nevertheless, the relationship between domestic cultivar distribution and agro-ecological zone could be observed in the UPGMA dendrogram. There are four main potato agro-ecological zones in China, including the northern single cropping zone, the southwest mixed cropping zone, the central double cropping zone and the southern winter cropping zone. Most of the cultivars that were developed in the same cropping zone clustered together. Most of the cultivars from the northern single cropping zone clustered in a large group. Among them, Kexin 19, Dongnong 304, Hushu 5, Kexin 2, Kexin 3, Kexin 13, Kexin 9, Chunshu 5, Hushu 1, Chaobai, Kexin 4, and Kexin 5, all from Northeast China, clustered in one subgroup. Surprisingly, pedigree information revealed that, most of these cultivars were descendants of Mira (one to two generations). Similarly, Qingshu 8, Longshu 1, Wensheng 4, Linshu 7, Xindaping, Ningshu 9, Ningshu 1, Shengli 1, Gaoyuan 7, Weishu 1, Yushu CA, Linshu 3, Weishu 8, Annong 5, Ningshu 6, Kangyi 1, Longshu 3, and Ningshu 7, all from Northwest China, clustered in another subgroup, and they were mostly progeny of the Polish cultivar

Epoka and German cultivars Schwalbe and Industria. Cultivars from the southwest mixed cropping zone also clustered in one group, for example, Yunshu 201, Yunshu 102, Yunshu 301, Yunshu 501, Yunshu 101, Chuanyu 6 and Weiyu 3. These cultivars were mainly derived from Mira and *neotuberosum*. Clustering analysis also showed that most of the cultivars that were bred by the same program clustered within the same group, for example, Kexin 2, Kexin 3, Kexin 4, Kexin 5, Kexin 9, Kexin 13, Kexin 14 and Kexin 19 from one breeding program clustered together; Yunshu 201, Yunshu 102, Yunshu 301, Yunshu 501 and Yunshu 101 from another breeding program clustered together.

## Discussion

Potato cultivar identification is of great importance for seed production, germplasm management and breeders' right protection. In this study, we successfully identified 20 polymorphic SSR markers to analyze the fingerprints of 217 potato

**Table 3** List of cultivars that produced unique alleles with certain SSR markers

Cultivar	Marker	Cultivar	Marker	Cultivar	Marker
Kexin 14	S118	Zhongshu 9	S25	Chunshu 4	S25
Kexin 19	S151	Zhongshu 13	S192	Weiyu 3	S184
Kexin 20	S180, S174	Jinshu 14	S7, S118	Liangshu 3	S174
Cooperation 001	S180, S184, S192	Jinshu 16	S170	Xindaping	S118
Cooperation 002	S174, S168	Yunshu 101	S180	Zhongdianhong	S180, S192
Cooperation 003	S7, S174	Yunshu 102	S184	Longshu 3	S170
Cooperation 88	S170, S168	Qinyu 30	S174	Mengshu 10	S151
Qingshu 5	S180	Qinyu 31	S151, S184, S168	Yanshu 4	S7
Qingshu 6	S7	Shuangfeng 4	S118	Jizhangshu 3	S118
Qingshu 7	S25	Shuangfeng 5	S151		
B76-16 (Xiaoyezi)	S168	Bashu 5	S118		

cultivars grown in China, and 11 of these markers enabled complete differentiation among all the cultivars. Our results indicate that SSR markers have enough power to differentiate the large number of potato cultivars in China. Moreover, genetic diversity analysis of 217 potato cultivars based on 20 polymorphic SSR markers indicates a narrow genetic background in these cultivars.

In our study, the number of alleles per locus (7–22) and PIC values (0.64–0.93) are comparable to the values reported by Ghislain et al. (2004); however, slight differences in these two studies could be attributed to the primer combinations used and the genetic background of the potato cultivars. The study by Ghislain et al. was done on 931 accessions in eight taxonomic groups of cultivated potato ranging from diploids to pentaploids, whereas, our study was done on 217 potato cultivars which were autotetraploid genotypes of different origin (the United States, Germany, Poland, the Netherlands, CIP, Canada, Czech Republic and domestic cultivars).

Previous studies have shown that a small number of SSR markers with high polymorphism could be used to distinguish large numbers of potato varieties. In this study, a subset of 11 SSR markers was sufficient to distinguish all 217 potato cultivars grown in China. Moisan-Thiery et al. (2005) successfully differentiated 286 potato cultivars produced in France with five SSR markers. Reid and Kerr (2007) developed six high polymorphic SSR markers that could differentiate more than 400 cultivars of European germplasm. Ghislain et al. (2009) also developed a potato genetic identification kit to differentiate 93.5% and 98.8% of the 742 landraces using 24 and 51 SSR markers, respectively. Karaagac et al. (2014) reported that 50 tetraploid genotypes could be differentiated with six SSR markers. These studies all indicated that SSR markers can efficiently identify potato germplasm materials at the molecular level. Further, we found that the number of unique alleles amplified by a SSR marker was not directly associated with the number of alleles detected or PIC values. For example, a maximum of six different unique alleles were amplified from six cultivars by marker S118 (Table 3). However, only 11 alleles for this marker were found in the 217 cultivars investigated and its PIC value was 0.87; these were moderate values for both number of alleles and PIC from among the 11 markers used. This finding was in agreement with results of Wang et al. (2003) on maize.

The analysis of genetic diversity of 217 potato cultivars indicated that the genetic base of potato germplasm in China was narrow. The vast majority of potato germplasm resources in China originated from abroad. There're 288 cultivars released in China during the period from 1950 to 2007, which can be mainly derived from cultivars or accessions from the United States, Germany, Poland, the Netherlands, and CIP. Among them, 100 cultivars were progeny of American cultivars including Katahdin, Early Rose, Houma, Wauseon, Pontiac, Triumph, Atlantic, and B76-16, which account for

34.7% of all the released cultivars in China during this time. 93 cultivars were progeny of German cultivars including Mira, Anemone, Schwalbe, Industria, Jubel, Merkur, Flava, Pepo, Amsel, Apta, and Mitterfrühe, accounting for 32.3%. 53 cultivars were progeny of Polish cultivar Epoka, accounting for 18.4%. Moreover, there're 20 and 4 cultivars that can be derived from the CIP resources and Netherlands cultivar Favorita, respectively. The other few cultivars were derived from other countries, domestic cultivars, or have unknown pedigree information. Sixteen cultivars we originally selected to screen for SSR marker polymorphism represented most of the germplasm of potato cultivars in China bred from 1950 to 2007.

In China, the use of a limited number of parental lines in the breeding programs may contribute to the narrow genetic base of improved cultivars. For example, six cultivars or accessions of Mira, Katahdin, Epoka, Anemone, Duozi bai, and B76-16 (Xiaoyezi) were found repeatedly in the parentage of 74 cultivars released before 1983, accounting for 68.8% of the total cultivars released in that period of time. In this study, cultivars released before 1983 were mostly clustered in one single group. The clustering together of most of the cultivars that came from the same cropping zone or the cultivars that were bred by the same program, strengthens the fact that few elite parental lines have been commonly and repeatedly used in potato breeding programs in China. In contrary, there were some closely related cultivars that did not cluster together in the same group. For example, Cultivar Linshu 2 and Linshu 3 were both bred from the same parents (B76-16 X Everest) but were placed in different groups. This may be attributed to non-specific fingerprints amplified during PCR or incorrect pedigree records for these cultivars.

The cultivated potato is a heterozygous autotetraploid species with four homologous sets of chromosomes ( $2n=4x=48$ ) (Gebhardt and Valkonen 2001). The selection of parental combinations is mainly dependent on morphological traits like growth habit, yield, resistance and processing quality, and less on pedigree information since the data is often scanty and sometimes incorrect (Braun and Wenzel 2005). However, morphological traits do not always provide a good measure of genetic makeup and may not accurately reveal genetic variation (Tanksley and McCouch 1997). Genetic relatedness measures based on molecular markers may not predict similarity in trait values or parental performance (Jansky et al. 2015). However, in order to improve heterotic effects, better knowledge about the degree of relationship between parents is required. In the present study, SSR markers were used to estimate genetic relationships in tetraploid potato cultivars with known pedigrees. The results showed that the cultivars with similar pedigrees grouped together in most cases. This is in agreement with the results of Braun and Wenzel (2005), who used AFLP and SSR

markers to evaluate genetic diversity in the tetraploid potato and to compare the findings with known pedigree information, and they found that in most cases the grouping of related genotypes and the known pedigree information was reflected well in dendrograms. Although our study found a narrow genetic base in the cultivars, breeders could try to choose distinct cultivars grouped in different subclasses as parents to introduce more diversity in the hybrids. In order to further improve the yield and quality of Chinese potato cultivars, it is necessary to extensively increase the genetic basis of the hybrid parents through the introduction and utilization of new germplasm resources.

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**Author's Contribution** LJ conceived the project; YD performed the molecular marker analysis; JL and JX conducted part of the experiment; SD collected the materials tested; CB and WP managed the field experiment; GL and JH contributed to the critical revision of the artwork and illustrations. All authors read and approved the final manuscript.

## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that there are no conflicts of interest.

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