www.czasopisma.pan.pl PAN www.journals.pan.pl

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 56/1: 16–27, 2014
DOI: 10.2478/abcsb-2014-0003

USING SSR TO EVALUATE THE GENETIC DIVERSITY OF POTATO CULTIVARS FROM YUNNAN PROVINCE (SW CHINA)

HONG LIAO AND HUACHUN GUO*

Tuber and Crop Research Institute, Yunnan Agricultural University, Kunming, P.R. China

Received September 13, 2012; revision accepted May 25, 2013

The genetic diversity of potato cultivars collected from Yunnan Province was evaluated using 24 pairs of SSR markers. SSR analysis of 24 pairs of primers showed varying degrees of polymorphism among the 85 cultivars: 297 of the 304 bands were polymorphic. The primers yielded between 5 (STM2028) and 19 (StI029) bands (mean 12). The ratio of polymorphic bands ranged from 83.33% to 100% (mean 97.75%). Polymorphism information content (PIC) varied from 69.31% to 93.67% (mean 86.47%). Genetic similarity ranged from 0.5987 to 0.7632, indicating relatively low genetic diversity in the potato cultivars from Yunnan Province. Cluster analysis by UPGMA and PCA clearly delineated the genetic relationships of all cultivars; 83 of the 85 cultivars could be discriminated by only two pairs of primers, STM0030 and STM1104. The high polymorphism and good resolution of the primers used in this study make them good tools for discriminating potato cultivars.

Key words: *Solanum tuberosum* L., SSR markers, genetic diversity, potato cultivars.

INTRODUCTION

Potato (Solanaceae) is an economically important food source consumed by people worldwide either as a non-grain staple or as a vegetable mainly, with global production of 323.5 million tons in 2007 (FAO Crops statistics database: http://faostat.fao.org/). The total planting area and production of potato in China account for a quarter of the world total, making it the most important country for potato production. Potato production in Yunnan Province is 9.2% of national production (Sui et al., 2008) and potato consumption in Yunnan Province is much higher than in other Chinese provinces due to its unique culture and dietary preferences. Development and exploitation of new potato cultivars are needed to increase crop productivity and meet the growing demand.

Development and utilization of genetic resources as well as germplasm conservation depend on an understanding of the genetic diversity and relationships between varieties from target regions. A number of molecular markers (e.g., RAPD, SCAR, SSR, ISSR) have been successfully used for evaluating genetic diversity, of which SSR has been used for pedigree (Sitther et al., 2012), genetic mapping (Song et al., 2005), and genetic diversity analysis (Baranski et al., 2012) because of its high polymorphism, codominance, simplicity and low cost. The first study using SSRs in potato characterized the genetic composition of anther-derived plants of a potato clone by searching in the published potato sequences, obtaining five polymorphic SSRs (Veilleux et al., 1995). Then Kawchuk et al. (1996) examined 252 *S. tuberosum* sequences, with 24 alleles observed, and the DNA products were used to establish a database for cultivar identification. Milbourne et al. (1997) used SSRs and three other PCR-based marker systems to examine genetic relationships in the potato gene pool, discriminating 16 cultivars. Other SSR sites have been found in the potato genome since then, including highly informative and user-friendly microsatellites (Ghislain et al., 2004). SSR markers have been used to identify French potato cultivars (Moisan-Thiery et al., 2005), discriminate potato germplasm in the INIA Chile breeding program (Mathias et al., 2007), develop a rapid SSR-based identification method for potato cultivars (Reid and Kerr, 2007), develop an SSR-based genetic identity kit for potato (Ghislain et al., 2009), and discriminate genetic relationships in Spanish potato cultivars (de Galarreta et al., 2011). This work has provided insights useful for germplasm management and discrimination of potato cultivars. In China and particularly in Yunnan

*e-mail: ynghc@126.com

PL ISSN 0001-5296

TABLE 1. Accessions used in this study

Code	Accession	Code	Accession	Code	Accession	Code	Accession
1	S03-259	23	711	45	Zhuanxinwu (landrace)	67	716
2	Aide53	24	S02-666	46	02-1-2	68	Yunshu 501
3	703	25	Zhongshu 901	47	S03-2689	69	02-006
4	P02-77-10	26	S03-2641	48	Ataboc	70	S02-741
5	Lishu 6	27	701	49	S03-3349	71	S03-3255
6	S02-367	28	02-028A	50	02-008	72	Yunshi 7
7	Xuanshu 2	29	YS03-3349	51	S03-2619	73	Lishu 7
8	Hezuo 88	30	Yunshi 5	52	802-17	74	705
9	S03-2406	31	S03-2744	53	Yunshu 101	75	714
10	Zhongshu 5	32	S03-3314	54	Xuanshu 3	76	Mira (German)
11	Yunshu 201	33	Yunshi 9	55	S03-1184	77	Russet Burbank (American)
12	S03-2751	34	S03-244	56	Hui-2	78	Y503-2744
13	Yunshi 3	35	S03-3309	57	P02-48-187	79	S03-1549
14	Ziyun 1	36	99-12-2	58	Keyi 85	80	719
15	S02-1424	37	04-017	59	YA03-4	81	B9908-11-1
16	N57	38	S03-3289	60	PI33	82	02-020
17	Shepody (Netherlands)	39	S03-4038	61	S03-2759	83	B11-01
18	723	40	S03-2369	62	S03-3276	84	717
19	Dianshu 6	41	Zhongshu 8	63	S01-198	85	Jima 2
20	Yunshu 301	42	Zhongshu 3	64	S02-336		
21	Jianchuanhong (landrace)	43	PB-08	65	1-4		
22	Hezuo 001	44	P02-52-2	66	S01-85		

Province there is a need for basic molecular information related to the genetic diversity and pedigree relationships of cultivated potato.

In this study we used SSR markers to analyze 85 potato varieties. The aims were (i) to evaluate the genetic diversity of potato cultivars in Yunnan Province, (ii) to determine the distribution of their genetic diversity in the province, and (iii) to screen SSR markers suitable for discriminating those potato varieties, with a view to improving their production and conservation.

MATERIALS AND METHODS

MATERIALS

Eighty-five potato cultivars (all tetraploid) were provided by the Tuber and Root Crops Research Institute (Yunnan Agricultural University, Kunming, China). Their names and code numbers are listed in Table 1. Ten healthy tubers of each variety were selected and planted in a greenhouse.

DNA EXTRACTION

Fresh tender leaves (~100 mg) from the plantlets were used to extract DNA according to the CTAB procedure of Moisan-Thiery et al. (2005). DNA quantity and quality were assessed on 0.8% (w/v) agarose gel with lambda DNA as concentration standard.

SSR PRIMERS

We selected 24 primers (Tab. 2) from those applied in other studies (Ghislain et al., 2004; Feingold et al., 2005; Reid and Kerr, 2007), based on high polymorphism and discrimination ability.

PCR AMPLIFICATION AND ELECTROPHORESIS

The PCR reactions was performed in a 20 µl volume containing 10×Taq DNA polymerase buffer, 2.0 µl 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 0.25 µM forward and reverse primer, 1.0 U Taq DNA polymerase (Tiangen, Beijing, China) and 50 ng genomic

TABLE 2. SSR primers and their characteristics

Primer name	SSR motif	Primer sequence	Chromosome location	Tm	Origin
STM1049	$(ATA)_6$	F:CTACCAGTTTGTTGATTGTGGTG R:AGGGACTTTAATTTGTTGGACG	I	57°C	Ghislain (2004)
STM2022	$(CAA)_3$…$(CAA)_3$	F:GCGTCAGCGATTTCAGTACTA R:TTCAGTCAACTCCTGTTGCG	II	53°C	Ghislain (2004)
STM1053	$(TA)_4(ATC)_5$	F: TCTCCCCATCTTAATGTTTC R: CAACACAGCATSCAGATCATC	III	53°C	Ghislain (2004)
STM3023a	$(GA)_9(GA)_8(GA)_4$	F: AAGCTGTTACTTGATTGCTGCA R:GTTCTGGCATTTCCATCTAGAGA	IV	50°C	Ghislain (2004)
STPoAc58	$(TA)_{13}$	F:TTGATGAAAGGAATGCAGCTTGTG R:ACGTTAAAGAAGTGAGAGTACGAC	V	57°C	Ghislain (2004)
STM0019a	$(AT)_7(GT)_{10}(AT)_4$ $(GT)_5(GC)_4(GT)_4$	F: AATAGGTGTACTGACTCTCAATG R:TTGAAGTAAAAGTCCTAGTATGTG	VI	47°C	Ghislain (2004)
STM2013	$(TCTA)_6$	F: TTCGGAATTACCCTCTGCC R: AAAAAAAGAACGCGCACG	VII	55°C	Ghislain (2004)
STIM1104	$(TCT)_5$	F:TGATTCTCTTGCCTACTGTAATCG R: CAAAGTGGTGTGAAGCTGTGA	VIII	57°C	Ghislain (2004)
STM3012	$(CT)_4$, $(CT)_8$	F: CAACTCAAACCAGAAGGCAAA R: GAGAAATGGGCACAAAAAACA	IX	57°C	Ghislain (2004)
STM1106	$(ATT)_{13}$	F: TCCAGCTGATTGGTTAGGTTG R: ATGCGAATCTACTCGTCATGG	X	55°C	Ghislain (2004)
STM0037	$(TC)_5(AC)_6AA$ $(AC)_7(AT)_4$	F:AATTTAACTTAGAAGATTAGTCTC R: ATTTGGTTGGGTATGATA	XI	53°C	Ghislain (2004)
STM0030	Compound $(GT/GC)(GT)_8$	F: AGAGATCGATGTAAAACACGT R: GTGGCATTTTGATGGATT	XII	53°C	Ghislain (2004)
StI004	(AAG)n	F:TTGATGAAAGGAATGCAGCTTGTG R:CAACTACAAGATTCCATCCACAG	VI	55°C	Feingold (2005)
StI017	(CAT)n(TAG)n (AAG)n	F:TATGGAAATTCCGGTGATGG R:GACGGTGACAAAGAGGAAGG	XI	64°C	Feingold (2005)
StI023	(GGC)n(GGT)n	F:GCGAATGACAGGACAAGAGG R:TGCCACTGCTACCATAACCA	X	55°C	Feingold (2005)
StI029	$(CA)_{imp}(TC)_{imp}$	F: GACTGGCTGACCCTGAACTC R:GACAAAATTACAGGAACTGCAAA	II	55°C	Feingold (2005)
StI033	(AGG)n	F:TGAGGGTTTTCAGAAAGGGA R:CATCCTTGCAACAACCTCCT	VII	64°C	Feingold (2005)
StI055	(AAG)n	F:CCGTTGATGGGATTGCACA R:TGATATTAACCATGGCAGCAGC	IV	55°C	Feingold (2005)
StI057	(AAG)n	F:CCTTGTAGAACAGCAGTGGTC R:TCCGCCAAGACTGATGCA	IX	55°C	Feingold (2005)
StI060	(ATA)n	F:ACTTCTGCATCTGGTGAAGC R:GGTCTGGATTCCCAGGTTG	III	55°C	Feingold (2005)
STM1031	$(AT)_{13}$	F:TGTGTTTGTTTTTCTGTAT R:AATTCTATCCTCATCTCTA	V	55°C	Ghislain(2004)
STM5148	$(GAA)_{17}$	F:TCTTCTTGATGACAGCTTCG R:ACCTCAGATAGTTGCCATGTCA	V	50℃	Reid (2007)
STM1024	$(TTG)_6$	F:ATACAGGACCTTAATTTCCCCAA R:TCAAAACCCAATTCAATCAAATC	VIII	50℃	Reid (2007)
STM2028	$(TAC)_5$…$(TA)_3$…$(CAT)_3$	F:TCTCACCAGCCGGAACAT R:AAGCTGCGGAAGTGATTTTG	XII	50℃	Reid (2007)
STM5136	$(AGA)_5$	F:GGGAAAAGGAAAAGCTCAA R:GTTTATATGAACCACCTCAGGCAC	I	50℃	Reid (2007)

DNA. The reaction system was run with a Biometra TProfessional thermocycler (Germany) using the following reaction conditions: 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 45 s at annealing temperature, 72°C for 1 min, with final extension at 72°C for 10 min, and storage at 4°C.

Before electrophoresis, 5 μl loading buffer was mixed with the PCR products and denatured at 95°C for 5 min, then transferred to an ice bath immediately until it was completely cooled for electrophoresis. Pre-electrophoresis was carried out without the PCR products on the gel at 450 V for 0.5 h. Then, with the power off, 5 μl of these denatured products were loaded into wells on 6% denatured polyacrylamide gel and then electrophoresis was run for 4 h at 450 V. After electrophoresis, the gel was stained by the modified silver staining method to obtain visible DNA bands, with 50 bp DNA marker (Takara, Dalian, China) as reference for product size. The staining buffer was freshly prepared as electrophoresis ended. After electrophoresis, the gel was placed into 1 L 0.2% $AgNO_3$ solution (10% ethanol, 0.5% acetic acid, 0.2% $AgNO_3$) to stain for 5–8 min. The stained gel was placed upright for 1–2 min to expel the extra staining buffer above the gel surface, and then developed in 1 L 3% developer (3% NaOH, 0.5% formaldehyde) for 10 min. With this method the gel does not need to be washed with deionized water, reducing staining time and cost; it makes the procedure easy to conduct in an ordinary lab.

SSR DATA ANALYSIS

Twenty-four pairs of SSR primers were employed to amplify the DNA of the 85 accessions. For each primer, amplification was performed twice to ensure stability and reproducibility. The SSR alleles were determined by size (bp) and scored 1 for present and 0 for absent at the same mobility. Size calculation used a 50 bp DNA marker (Takara, Dalian, China). Simple matching (SM) genetic similarity coefficients were computed from the matrix of scored 0–1 results, and were used to infer the genetic relationships between the cultivars in a sequential agglomerative hierarchical nesting (SHAN) module, using the unweighted pair-group method with arithmetic mean (UPGMA). The similarity coefficient matrix was used for principal component analysis (PCA). All of these analyses used NTSYS ver. 2.11C and followed methods described by Dice (1945). In addition, the percentage of polymorphic sites was calculated by the formula:

$$P = (k/n) \times 100\%,$$

where k is the number of polymorphic sites and n is the number of sites examined. The polymorphism information content (PIC) for each SSR locus was calculated by the formula

$$PIC = 1 - \sum_{1}^{i} f_i^2$$

where f_i is the relative frequency of the i^{th} allele of the SSR loci (Nei, 1973). The rate of distinguishing cultivars by cluster (RDCC) of each pair of primers for the detected accessions can be expressed by the formula

$$RDCC = (N-Ni)/N$$

in which *Ni* is the number of indistinguishable cultivars and N is the total number of accessions (Liu et al., 2010). Taking all the accessions in this research as a whole population, Nei's (1973) genetic diversity index (h) and Shannon's Information index (I) (Peet, 1975) were calculated using POPGENE ver. 1.32 (Yeh et al., 1997) to evaluate the genetic diversity of the whole set.

RESULTS

DISCRIMINATORY POWER OF SSR PRIMERS

The products of the 24 primer pairs ranged in size from 70 to 590 bp. Of the 304 bands produced, 297 (97.70%) were polymorphic and only 7 were monomorphic. The primer pairs produced 5–19 bands each (mean 12). Primer StI029 gave the most bands, and primer STM2028 the fewest. The percentage of polymorphic sites ranged from 83.33% to 100% (mean 93.46%). The polymorphism information content (PIC) differed between SSR primers, highest for StI029 (93.67%) and lowest for STM2028 (69.31%); mean PIC was 86.47% (Tab. 3). This means that all the primers selected were good indicators of the genetic diversity of the accessions.

The rate of distinguishing cultivars by cluster (RDCC) and maximum genetic similarity are listed in Table 4. The dendrograms of three pairs of these primers (STM2028, STM1049, STM3012) were abandoned during the clustering process because there were more than 25 nodes using NTSYS software. We evaluated these primers based on the criterion that the primer with higher genetic similarity and lower RDCC is deemed to have low discriminatory power (Liu et al., 2010). Primer STM0030 with 95.65% RDCC was the best primer; it distinguished 83 of the 85 varieties, leaving only 2 varieties unseparated. Primers StI029, STM5136, STM0019a showed less discrimination, and primer STM2022 the least (9.41%). The difference in RDCC between the primer pairs is shown in dendrograms (Fig. 1a, b). The electrophoretogram showed clear bands for every well (Fig. 2).

GENETIC DIVERSITY AMONG THE 85 ACCESSIONS

To assess the diversity of the 85 Yunnan potato varieties we calculated simple matching (SM) genetic similarity coefficients using NTSYS. SM was lowest (0.5592) between accession 53 (Yunshu 101) and accession 13 (Yunshi 3), and highest (0.9342) between accession 41 (Zhongshu 8) and accession

TABLE 3. Results of applying 24 SSR primers to distinguish Yunnan Province potato cultivars

Primer name	Range	No. of alleles	No. of polymorphic alleles	Ratio of polymorphism %	PIC %
STM1049	184–400bp	7	6	85.71	78.53
STM2022	184–325bp	8	8	100.00	78.67
STM1053	168–355bp	13	12	92.31	87.51
STM3023a	169–500bp	12	11	91.67	89.94
STPoAc58	240–550bp	14	14	100.00	90.62
STM0019a	85–220bp	14	14	100.00	90.86
STM2013	145–350bp	12	12	100.00	89.92
STM1104	150–295bp	15	15	100.00	91.18
STM3012	150–500bp	7	6	85.71	79.11
STM1106	140–350bp	14	14	100.00	88.60
STM0037	70–190bp	14	14	100.00	86.98
STM0030	100–405bp	18	18	100.00	93.32
STM1031	265–500bp	10	10	100.00	79.04
STM2028	288–410bp	5	5	100.00	69.31
STM1024	140–325bp	12	10	83.33	84.55
STM5136	215–550bp	19	19	100.00	91.73
STM5148	405–590bp	7	7	100.00	83.31
StI004	70–153bp	14	14	100.00	88.71
StI017	150–400bp	14	14	100.00	87.93
StI023	180–520bp	17	17	100.00	88.50
StI029	120–380bp	19	19	100.00	93.67
StI055	210–500bp	14	13	92.86	87.90
StI057	180–520bp	16	16	100.00	90.83
StI060	150–400bp	9	9	100.00	84.58
Total	—	304	297	—	—
Mean	—	12.67	12.38	93.46%	86.47%

42 (Zhongshu 3); mean SM was 0.6756. The frequency distribution bar chart (Fig. 3) was produced using SPSS 17.0 according to the 3570 genetic similarity coefficients obtained using NTSYS, with 0.0165 for group interval. A trend toward a standard normal distribution was observed among the 85 Yunnan potato varieties.

Genetic similarity of all individuals ranged from 0.5592 to 0.9342, with mean similarity of 0.6756, suggesting that potato cultivars in Yunnan had close genetic relationships with each other and genetic diversity was relatively low.

GENETIC RELATIONSHIPS OF 85 ACCESSIONS SHOWED BY DENDROGRAM AND PCA ANALYSIS

A dendrogram was constructed based on cluster analysis of Nei's (1978) unbiased genetic similarity of SSR alleles among the cultivars (Fig. 4). With a similarity coefficient cut-off point of 0.664, five groups were differentiated (I–V, Tab. 5). The first group contains most of the cultivars and two subgroups. The first subgroup includes 19 cultivars. In this subgroup, accessions 13 (Yunshi 3), 30 (Yunshi 5) and 72 (Yunshi 7) assemble in a small subcluster with relatively high similarity coefficients, probably due to maternal or paternal kinship, though no information was found about the origins of the three accessions. The similarity coefficient was highest (0.9342) for accessions 41 and 42. The second subgroup consists of 55 cultivars, including nearly all of the S02-serials (e.g., S02–367, S02–1424, S02–666) and S03-serials (e.g., S03–259, S03–2406, S03–2751), and all of the 7-serials (e.g., 703, 723, 711). The second group contains only two accessions (nos. 4, 5). The third group consists of five

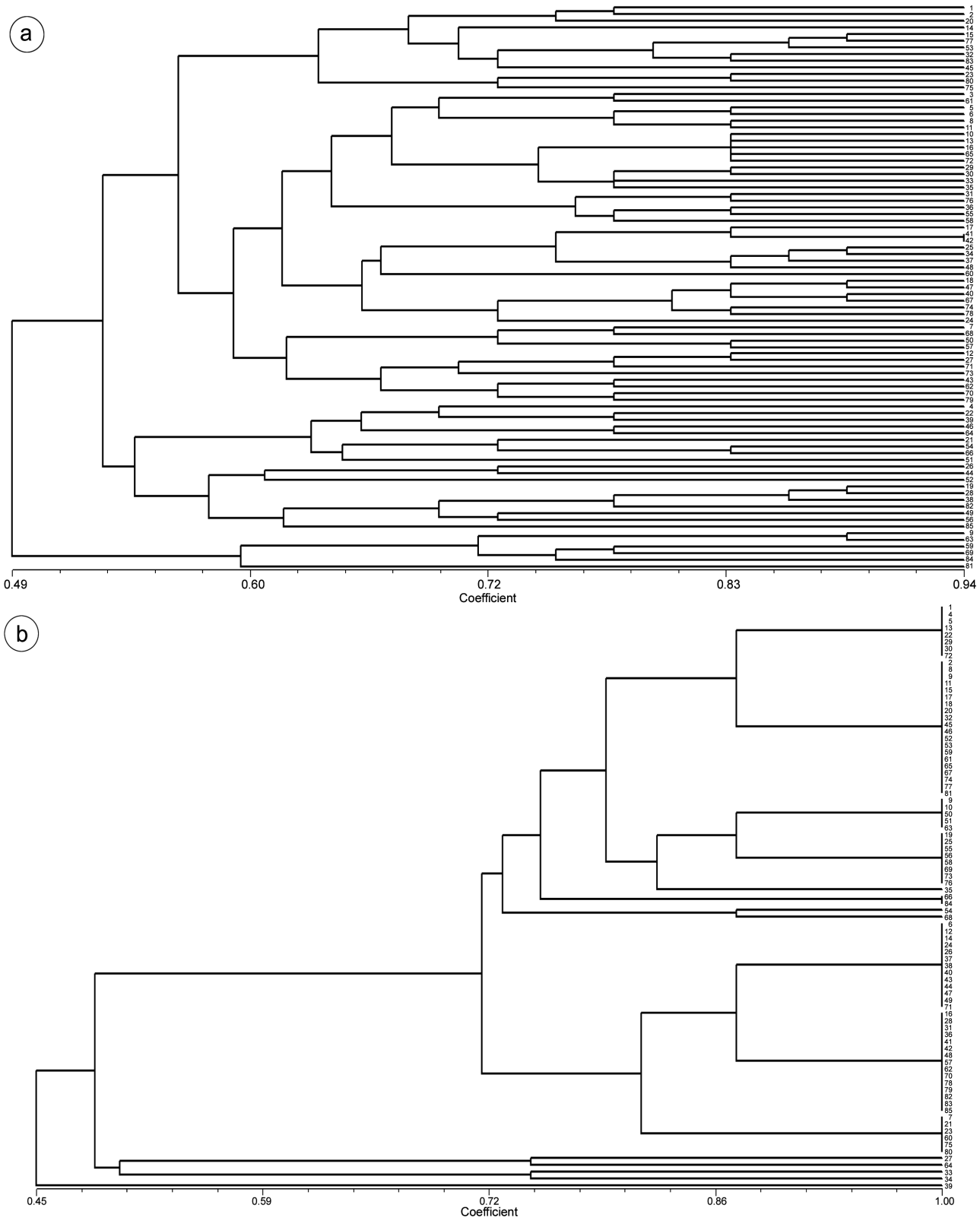

Fig. 1. UPGMA dendrogram of all accessions based on primer STM0030 (**a**) and primer STM2022 (**b**).

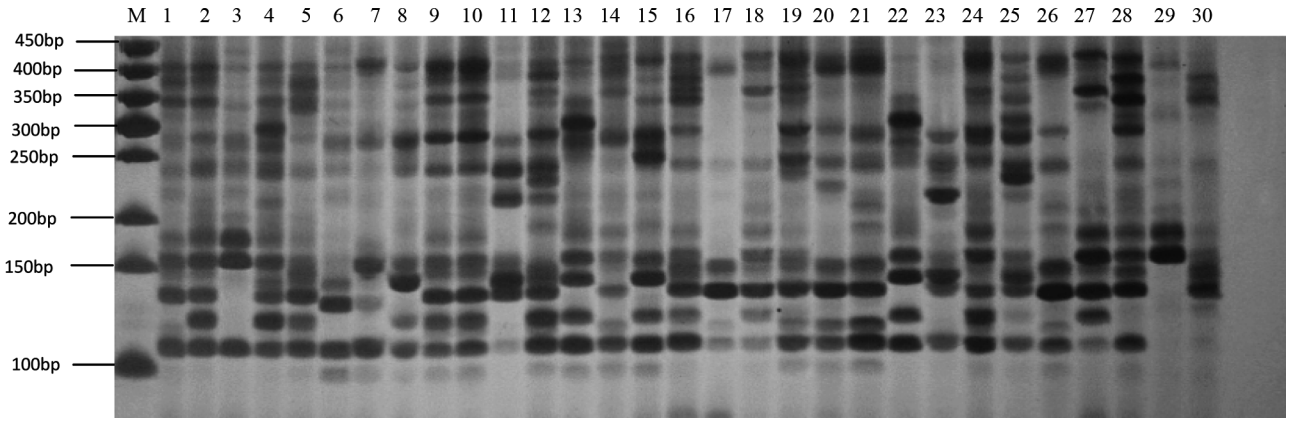

Fig. 2. PCR results based on primer STM0030.

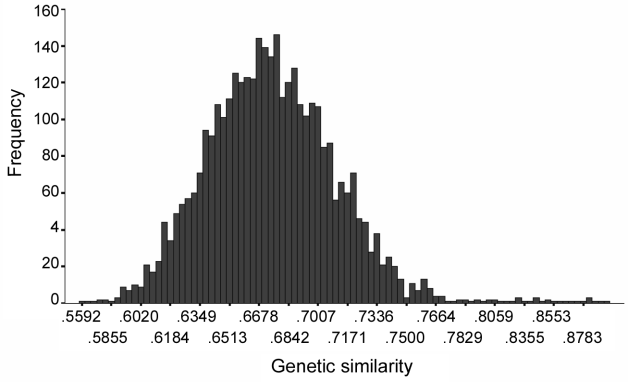

Fig. 3. Frequency distribution of SSR genetic similarity coefficients among 85 accessions.

accessions: nos. 2, 15, 53, 52, 63. One accession (no. 11) comprises group IV. The three accessions left (nos. 33, 34, 54) form the last group. Table 5 lists the cultivars in every group.

DISCUSSION

GENETIC DIVERSITY OF POTATO CULTIVARS IN YUNNAN PROVINCE

The genetic diversity coefficients in this work formed a normal distribution. Zhao et al. (2010) obtained a normal distribution of genetic diversity coefficients for 30 Japonica rice varieties.

Our results show relatively low genetic diversity among 85 Yunnan potato cultivars. Of the 3570 genetic similarity coefficients, 3511 (98.35%) ranged between 0.5987 and 0.7632. Eleven of the values were lower than 0.5987, and 48 higher than 0.7632. The mean similarity coefficient from DNA fingerprinting was 0.6750. Yunnan Province is a mountainous area and not all the potato germplasm there has been sampled. Genetic diversity results depend on the completeness of collections. Particularly in underdeveloped regions it is important to conserve and evaluate germplasm because many unique alleles can be found in locations where anthropopression is low.

In new collections from the Santa Catalina Mountains, Bamberg and Del Rio (2011) found three times as many unique alleles as in previous collections from that location, demonstrating that the remote as well as the easily accessible places in mountain ranges need to be searched for unique alleles. The genetic diversity of the potato germplasm of nearly all the provinces of China is relatively low (e.g., inner Mongolia, Gansu, Qinghai-Teng et al., 2009; Guizhou-Li et al., 2010; Sichuan-Tang et al., 2011). The underlying reason for this may be that breeders usually make use of several elite lines to meet the demands of the potato industry, which requires special varieties for chips, crisps, starch processing and retail sale. This trend will reduce the genetic diversity of potato cultivars, affecting their adaptation and thus their survival and sustainability. The dearth of knowledge about the genetic diversity and genetic relationships of these accessions limits their use in potato breeding (Lung'aho et al., 2011). The number of potato lines needs to be increased, making use of excellent accessions such as landraces and wild resources. Another resource is the diverse germplasm from the International Potato Center (CIP) in Lima, Peru. Yunnan Province has distinctive native potato varieties such as Zhuanxinwu and Jianchuanhong. These are colored potato landraces of outstanding quality. Other distinctive cultivars may be produced with the use of these two cultivars.

UNIFORMITY BETWEEN SSR DENDROGRAM AND PEDIGREE

In terms of pedigree, both accession 55 (S03-1184) and accession 11 (Yunshu 201) are progenies of the

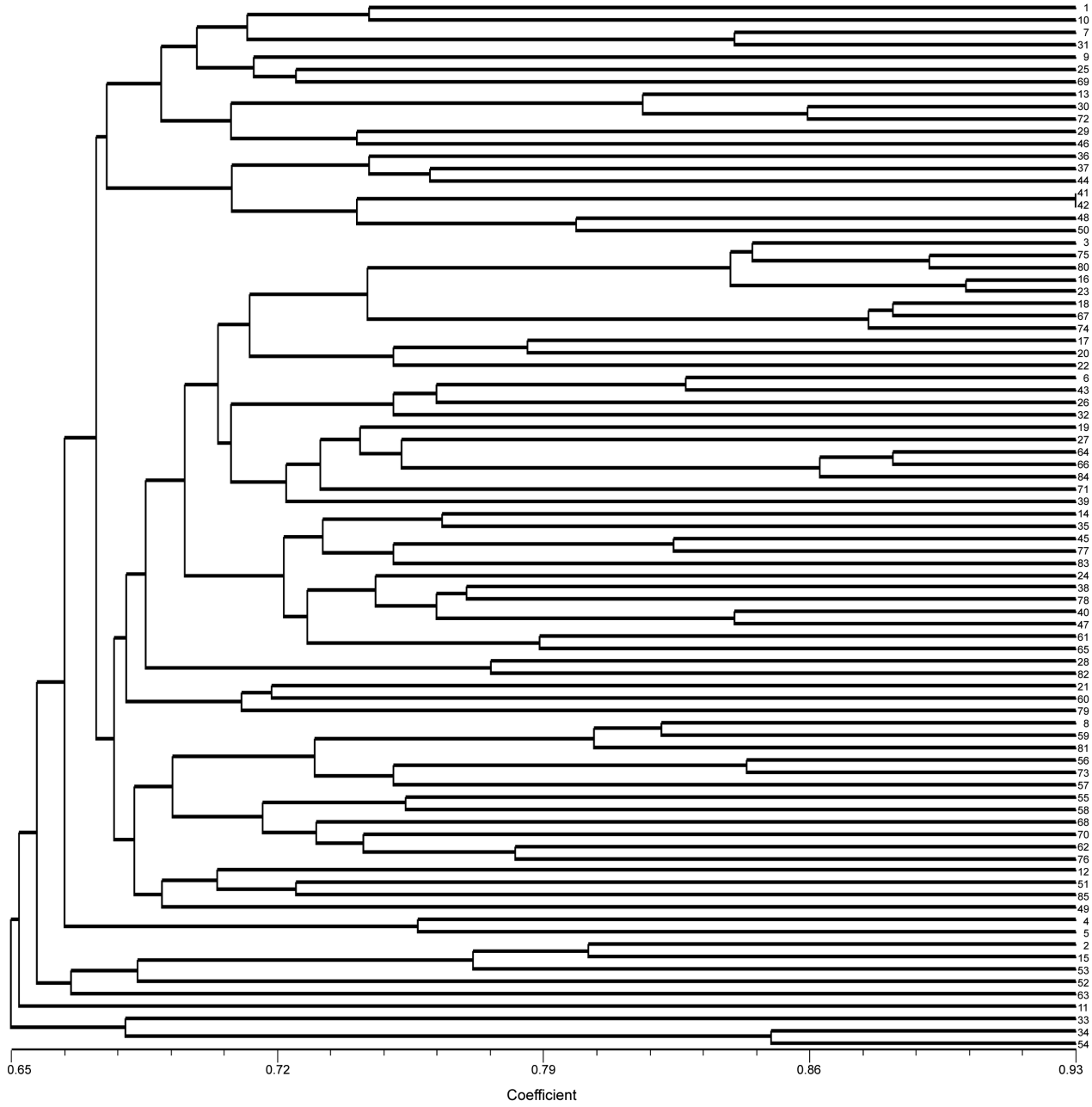

Fig. 4. Dendrogram of 85 accessions based on 24 pairs of primers.

hybridized combination S95–105 Neishu 7 obtained through individual plant selection and asexual generation. Why are they in different groups? This may due to the high genetic variation among true seed offspring of the potato, as reflected in the observed lack of SSR uniformity (Ai et al., personal communication) and ISSR uniformity (Ai et al., 2010) between true seed offspring and their maternal plants. There are notable differences in genetic background among the true seeds of selfing in potato, letting alone progenies of cross combination. In a study of cultivars from the same institute, however, Duan (unpubl. data) found that Yunshu 101, Yunshu 201, Yunshu 301 and Yunshu 501 bred by the Yunnan Academy of Agricultural Sciences clustered in one subgroup.

In our work, accessions 41 and 42 gave the highest similarity coefficient, 0.9342. Accession 41 (Zhongshu 8) is the progeny of W953 FL475, while accession 42 (Zhongshu 3) is the progeny of Jingfeng 1×BF67A (Duan, unpubl. data). Thus they

TABLE 4. Ability of 24 SSR primers to distinguish Yunnan Province potato cultivars

Primer	Numbers of distinguishable cultivars (Nd)	Numbers of indistinguishable cultivars (Ni)	RDCC %	Max GS
STM1049	—	—	—	1.0000
STM2022	8	77	9.41	1.0000
STM1053	19	66	22.35	0.9231
STM3023a	46	39	54.12	0.9167
STPoAc58	50	35	58.82	0.9286
STM0019a	57	28	67.06	1.0000
STM2013	49	36	57.65	1.0000
STM1104	54	31	63.53	1.0000
STM3012	—	—	—	1.0000
STM1106	23	61	27.06	1.0000
STM0037	47	41	55.29	1.0000
STM0030	83	2	97.65	1.0000
STM1031	14	71	16.47	1.0000
STM2028	—	—	—	1.0000
STM1024	12	73	14.12	1.0000
STM5136	65	20	76.47	1.0000
STM5148	26	59	30.59	1.0000
StI004	31	54	36.47	1.0000
StI017	36	49	42.35	1.0000
StI023	41	44	48.24	1.0000
StI029	63	22	74.12	1.0000
StI055	30	55	35.29	1.0000
StI057	53	32	63.356	1.0000
StI060	12	73	14.12	1.0000

Max GS – Maximum genetic similarity; RDCC – Rate of distinguishing cultivars by cluste

TABLE 5. Cultivars included in all UPGMA clusters

Cluser code	No. of cultivars	Cultivars contained in each group
I	74	First subgroup (19 accessions): S03-259, Zhong 5, Xuanshu 2, S03-2744, S03-2406, Zhong 901, 02-006, Yunshi 3, Yunshi 5, Yunshi 7, YS03-3349, 02-1-2, 99-12-2, 04-017, P02-52-2, Zhong 8, Zhong 3, Ataboc, 02-008 Second subgroup (55 accessions): 703, 714, 719, N57, 711, 723, 716, 705, Shepody Yunshu 301, Hezuo 001, S03-367, PB-08, S03-2641, S03-3314, Dianshu 6, 701, S02-336, S01-85, 717, S03-3255, S03-4038, Ziyun 1, S03-3309, Zhuan xin wu, Russet Burbank, B11-01, S02-666, S03-3289, Y503-2744, S03-2369, S03-2689, S03-2759, 1-4, 02-028A, 02-020, Jian chuan hong, PI33, S03-1549, Hezuo 88, YA03-4, B9908-11-1, Hui-2, Lishu 7, P02-48-187, S03-1184, Keyi 85, Yunshu 501, S02-741, S03-3276, Mira, S03-2751, S03-2619, Jiama 2, S03-3349
II	2	P02-77-10, Lishu 6
III	5	Aide 53, S02-1424, Yunshu 101, 820-17, S01-198
IV	1	Yunshu 201
V	3	Yunshi 9, S03-244, Xuanshu 3

differ in pedigree. Why are these two accessions so similar at the 24 loci? More investigation is needed, but on the principle that when 2 or more loci differ between two cultivars, which is equal to genetic similarity lower than 0.95, then these two cultivars can be identified as different (Zhao and Wang, 2009). Accessions 41 and 42 should be identified as two different cultivars despite their high genetic similarity (0.9342). Genetic similarity was lowest between accessions 53 and 13, possibly due to differences in pedigree.The selection of primers can also affect the result. In this research we found that the dendrogram trees differed considerably depending on which results of different primers or primer combinations were used (data not shown). A cultivar may be in a cluster with some cultivars in one dendrogram, and in another dendrogram based on different primer combinations it may cluster with other cultivars. For example, accessions 41 and 42 were in the same cluster and had the highest genetic similarity coefficient, but with other primer combinations they were in two different clusters and had a lower coefficient. The selection of primers is critical to the accuracy of potato cultivar identification. China has not yet established a national standard set of SSR markers to verify the genuineness and purity of potato cultivars.

THE POTENTIAL OF SSR TECHNIQUE FOR CONSTRUCTION OF CORE COLLECTIONS, DNA FINGERPRINTING AND CULTIVAR IDENTIFICATION

Here we demonstrated the usefulness of the primers employed in this study for potato germplasm discrimination.

The application of SSR technique for potato genetic analysis began with a study of anther-derived potato (Veilleux et al., 1995) and was quickly followed by other research. Ghislain et al. (2006) from CIP showed the superior polymorphism detection power of nSSR as compared to RAPD markers. SSR and SNP techniques are presently considered the best methods to use in constructing a core collection and fingerprinting cultivars. SSR technique has become the most common method used to construct a cultivar database, because of its simplicity, extensive distribution, codominance, low cost and high polymorphism. Reid and Kerr (2007) used SSR technique to identify cultivars by screening a number of microsatellite markers, producing a set of six that could be used to differentiate over 400 cultivars. We used 24 pairs of primers to analyze genetic diversity. In this work, one powerful primer (STM0030) identified 83 of the 85 cultivars, leaving just two (Zhongshu 3, Zhongshu 8), which were differentiated by primer STM1104. Theoretically, the combination of these two primers could identify all the cultivars. However, when these two primers were combined, two other accessions were not separated by the data these primers produced (accessions 18 and 76); that is to say, more primers need to be screened to find primers with superior discriminatory power. Several years ago the government of China considered drawing up a national standard of "Genuineness and Purity Verification of Potato Seed-Tuber SSR Molecular Markers", in which 12 pairs of primers (included among the primers used in our research) were established as standard primers. Under any conditions, including repeatedly optimized reaction conditions and reaction systems, it was difficult to get clear and well distinguishable bands using primer STM0037, listed in that standard. Perhaps more attention was focused getting an on even distribution on the 12 chromosomes, ignoring the quality of the reaction products of these primers. To complete the national standard and provide a formal means of identifying, documenting and monitoring potato germplasm, primers such as StI017, used in our research and located on the same chromosome as STM0037, can be employed to replace it. Ghislain et al. (2009) developed a potato genetic identity kit able to discriminate 93.5% of 742 landraces. Such a kit might be developed for research in China to facilitate communication between different labs and institutes, or a worldwide PGI kit could be devised for cooperation between many nations. The origins of the potato cultivars must be taken into consideration, however. The selection of SSR primers ought to be based on their origin, since with the increase in the number of potato cultivar entries there is a risk of identical molecular patterns appearing for distinct cultivars (Moisan-Thiery et al., 2005). In China itself the origin of potato cultivars is debatable due to the lack of extensive records about the introduction of potato in China. Much work on the origin and dissemination of potato remains to be done.

The primers used in this study include the six pairs of primers used by Reid and Kerr (2007). We found that the six primers could differentiate 83 of the 85 cultivars; only two cultivars (accessions 75 and 80) clustered at a similarity coefficient of 0.98 (data not shown), demonstrating the universality of those primers and their usefulness for collaboration between different institutes. Capillary electrophoresis technology developed in recent years has the advantage of high throughput, high accuracy, and automatic gel-pouring, sampling, electrophoresis and data collection. It is highly efficient and avoids human error, especially band-reading error, improving the stability and reproducibility of results. Integrating SSR and capillary electrophoresis saves time and energy consumption, and avoids handling of toxic polyacrylamide gel and silver stain (Zhao and Wang, 2009). Reid and Kerr (2007) used capillary electrophoresis technique to produce a rapid method of potato cultivar identification. For

fingerprinting and identification of potato cultivars a large number of SSR primers for capillary electrophoresis need to be the developed.

The main sources of SSR primers lie in related literature, the primers of related species, primers designed according to the flanking sequences of SSR loci by screening from the genomic library of the studied groups, and database searching (Zhou et al., 2008). Among these methods, database searching is the most direct and accurate way of developing primers. Visser et al. (2009) stated that "the potato genome sequence will provide a major boost to gaining a better understanding of potato trait biology, underpinning future breeding efforts." The Potato Genome Sequence Consortium (PGSC), bringing together scientists from many nations including China (Potato Genome Sequencing Consortium, 2011) has worked on potato genome sequencing and annotation, providing information for primer design through database searching; the organization provides a platform for genetic improvement of the disease resistance, stress resistance, cultivar quality and yield of potato. Annotation of the potato genome sequence will reveal more genes and their functions and will pave the way for the development of functional markers. Functional markers can be used to ascertain the presence or absence of certain genes without further verification, greatly simplifying the task of identifying alleles.

AUTHORS' CONTRIBUTIONS

Both authors contributed to this paper: HG designed research; HL performed research, analyzed data; HL. and HG interpreted the results and wrote the paper. Both authors approved the manuscript.

ACKNOWLEDGEMENTS

This research was supported by Modern Agricultural Technology System special funds (CARS-10), China. We thank the members of the Tuber and Crop Research Institute, Yunnan Agricultural University, China, for their help with plant material collection, cultivation, and harvesting, and Dr. G. Hua Chun for valuable advice and consultation.

REFERENCES

Ai XM, Wang Q, Guo HC, and Zhang XY. 2010. Genetic diversity analysis of 'Zhuanxinwu' and its seedling populations of Yunnan potato landraces by ISSR. *Molecular Plant Breeding* 8: 920–924.

Bamberg J, and del Rio A. 2011. Diversity relationships in tetraploid wild potato native to the USA. *American Journal of Potato Research* 88: 29.

Baranski R, Kaul A M, Nothnagel T, Cavagnaro PF, Simon PW, and Grzebelus D. 2012. Genetic diversity of carrot (*Daucus carota* L.) cultivars revealed by analysis of SSR loci. *Genetic Resources and Crop Evolution* 59: 163–170.

de Galarreta JIR, Barandalla L, Rios DJ, Lopez R, and Ritter E. 2011. Genetic relationships among local potato cultivars from Spain using SSR markers. *Genetic Resources and Crop Evolution* 58: 383–395.

Dice LR. 1945. Measures of the amount of ecologic association between species. *Ecology* 26: 297–302.

Feingold S, Lloyd J, Norero N, Bonierbale M, and Lorenzen J. 2005. Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). *Theoretical and Applied Genetics* 111: 456–466.

Ghislain M, Andrade D, Rodriguez F, Hijmans R, and Spooner D. 2006. Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja group using RAPDs and nuclear SSRs. *Theoretical and Applied Genetics* 113: 1515–1527.

Ghislain M, Nunez J, del Rosario Herrera M, Pignataro J, Guzman F, Bonierbale M, and Spooner D. 2009. Robust and highly informative microsatellite-based genetic identity kit for potato. *Molecular Breeding* 23: 377–388.

Ghislain M, Spooner D, Rodriguez F, Villamon F, Nunez J, Vasquez C, Waugh R, and Bonierbale M. 2004. Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theoretical and Applied Genetics* 108: 881–890.

Kawchuk L, Lynch D, Thomas J, Penner B, Sillito D, and Kulcsar F. 1996. Characterization of *Solanum tuberosum* simple sequence repeats and application to potato cultivar identification. *American Potato Journal* 73: 325–336.

Li F, Deng K, Duan Y, Liu J, Fan S, and Lei Z. 2010. Genetic analysis and finger-print establishment of eight new potato varieties. *Guizhou Agricultural Science* 38: 9–10.

Liu X, Ma L, Chen X, Ying X, Cai Q, Liu J, and Wu C. 2010. Establishment of DNA fingerprint ID in sugarcane cultivars in Yunnan, China. *Acta Agronomica Sinica* 36: 202–210.

Liu X, Mao J, Lu X, Ma L, Aitken K, Jackson P, Cai Q, and Fan Y. 2010. Construction of molecular genetic linkage map of sugarcane based on SSR and AFLP markers. *Acta Agronomica Sinica* 36: 177–183.

Lung'aho C, Chemining'wa GN, Fu YB, Shibairo SI, Hutchinson MJ, and Paniagua HG. 2011. Genetic diversity of Kenyan potato germplasm revealed by simple sequence repeat markers. *American Journal of Potato Research* 88: 424–434.

Mathias MR, Sagredo BD, and Kalazich JB. 2007. Use of SSR markers to identify potato germplasm in the INIA Chile breeding program. *Agricultura Tecnica* 67: 3–15.

Milbourne D, Meyer R, Bradshaw J, Baird E, Bonar N, Provan J, Powell W, and Waugh R. 1997. Comparison of PCR-based marker systems for the analysis of genetic relationships in cultivated potato. *Molecular Breeding* 3: 127–136.

Moisan-Thiery M, Marhadour S, Kerlan M, DessennE N, Perramant M, Gokelaere T, and le Hingrat Y. 2005. Potato cultivar identification using simple sequence repeats markers (SSR). *Potato Research* 48: 191–200.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences* 70: 3321–3323.

Peet RK. 1975. Relative diversity indices. *Ecology* 56: 496–498.

Reid A, and Kerr E. 2007. A rapid simple sequence repeat (SSR)-based identification method for potato cultivars. *Plant Genetic Resources* 5: 7–13.

Sitther V, Zhang D, Dhekney SA, Harris DL, Yadav AK, and Okie WR. 2012. Cultivar identification, pedigree verification, and diversity analysis among peach cultivars based on simple sequence repeat markers. *Journal of the American Society for Horticultural Science* 137: 114–121.

Song Q, Shi J, Singh S, Fickus E, Costa J, Lewis J, Gill B, Ward R, and Cregan P. 2005. Development and mapping of microsatellite (SSR) markers in wheat. *Theoretical and Applied Genetics* 110: 550–560.

Sui Q, LI X, and Yang W. 2008. Analysis of potato production in China. Southwest China *Journal of Agricultural Science* 21: 1182–1188.

Tang M, He W, Hu J, Wang K, Chen P, Mou W, and Yang L. 2011. Genetic diversity of potato (*Solanum tuberosum* L.) in Sichuan Province. *Southwest China Journal of Agricultural Science* 23: 1805–1808.

Teng C, Zhang Y, and Zhang F. 2009. Genetic diversity of leading potato cultivars in Qinghai Province revealed by SSR. *Molecular Plant Breeding* 7: 555–561.

Veilleux RE, Shen LY, and PAZ MM. 1995. Analysis of the genetic composition of anther-derived potato by randomly amplified polymorphic DNA and simple sequence repeats. *Genome* 38: 1153–1162.

Visser R, Bachem C, de Boer J, Bryan G, Chakrabati S, Feingold S, Gromadka R, van Ham R, Huang S, and Jacobs J. 2009. Sequencing the potato genome: outline and first results to come from the elucidation of the sequence of the world's third most important food crop. A*merican Journal of Potato Research* 86: 417–429.

Potato Genome Sequencing Consortium. 2011. Genome sequence and analysis of the tuber crop potato. *Nature* 475: 189–195.

Yeh FC, Yang R, Boyle TBJ, Ye Z, and Mao JX. 1997. POPGENE, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Zhao J, and Wang F. 2009. *Application and Research on DNA Fingerprinting of Maize Cultivars*, Chapter 2. Chinese Agricultural Science and Technology Press, Beijing.

Zhao Q, Zhang Y, Zhu Z, Zhao L, Chen T, and Wang C. 2010. Analysis on genetic diversity of 30 Japonica rice varieties using SSR markers. *Journal of Plant Genetic Resources* 11: 218–223.

Zhou Y, Yang Q, and Zhang G. 2008. *Biological Genetic Markers and Application*, Chapter 6. Chemical Industry Press, Beijing.