

Germplasm Evaluation and Molecular Selection of Potato (*Solanum tuberosum* L.) Cultivars with Disease Resistance in China

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Abstract

Li W.L., Guo W., Xiao J.P., Bai L., Guo H.C. (2017): Germplasm evaluation and molecular selection of potato (*Solanum tuberosum* L.) cultivars with disease resistance in China. Czech J. Genet. Plant Breed., 53: 114–121.

Foreground and background selections are two important aspects that need to be carefully considered by plant breeders during field selection. In this article, we used 7 disease resistance markers, including four late blight and three potato virus disease resistance gene markers, and 12 microsatellite markers to evaluate the disease resistance and genetic diversity of 76 potato cultivars which were collected from 15 provinces of China. The foreground selection results showed that a number of materials, clustered separately, contained more than two late blight resistance markers or combined late blight and virus disease resistance gene markers. Many of them were collected from the southwest of China. Additionally, the genetic backgrounds of all cultivars were relatively narrow and a limited number of cultivars (15.8%) contained both potato late blight and potato virus Y resistance markers. Also, only two accessions (Yunshu 103 and Lishu 7) contained both late blight and potato virus X resistance markers. In conclusion, this comprehensive evaluation of genetic resources will shed the light on potato disease resistance breeding in the future.

Keywords: genetic diversity; potato late blight resistance; potato virus X resistance, potato virus Y resistance; SSR markers

Potato late blight (PLB) and viruses are the two most disastrous diseases on potato (*Solanum tuberosum* L.) (LAWSON *et al.* 1990; KAMOUN 2001; WHITE & SHAW 2010). Developing new cultivars with desirable disease resistances provides an environmentally friendly and cost efficient solution (OJIAMBO *et al.* 2000; GEBHARDT & VALKONEN 2001). However, significant genetic improvement in autotetraploid potato, whose inheritance is complicated, takes a lot of time (SOLOMON-BLACKBURN & BARKER 2001). Fortunately, this situation has been improved by the application of molecular tools in plant breeding. Among them, marker-assisted selection (MAS) can directly select the specific genomic regions by using molecular markers which are linked to the traits of interest (BABU *et al.* 2004). The advantage of this method is selection of genotypes, instead of phenotypes, which significantly reduces the time for new

cultivar development and labour cost. Previously, many resistance genes have been introgressed into cultivated potato from different sources and the corresponding PCR-based markers have also been developed, which might facilitate tracing and combining them in a potato breeding process (BARONE 2004; ORTEGA & LOPEZ-VIZCON 2012). Here, in the present study, three markers developed from the actual gene sequence (markers of the resistance genes *R1*, *R3a*, *R3b*) and four markers closely linked to underlying genes (markers of the resistance genes *R2*, *Ry_{chc}*, *Ry_{adg}*, *Rx1*) (KASAI *et al.* 2000; BALLVORA *et al.* 2002; HUANG *et al.* 2005; LI *et al.* 2011; MORI *et al.* 2011, 2012) were used to evaluate potato late blight, potato virus Y (PVY) and potato virus X (PVX) resistances of 76 potato cultivars in China.

Broad adaptability refers to the ability of living in various biotic and abiotic conditions and has a broad

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genetic basis for resilience. It is also a crucial aspect which should be considered during new cultivar breeding and development. However, plant breeding usually reduces genetic diversity within germplasm which could seriously jeopardize the sustainable improvement of crops (IQBAL *et al.* 2009). Effective detection and utilization of different genetic resources within the germplasms have been the primary activities of breeders for crop improvement. Taking the advantage of molecular tools, breeders can easily systematically evaluate genetic diversity which exists in the germplasm collections and quickly target the best parents as well as design crossing schemes. In potato, SSR markers have been shown to be highly polymorphic and well discriminant (GHISLAIN *et al.* 2004). In the present study we evaluated the genetic diversity of 76 elite Chinese potato cultivars, including 35 cultivars from southwest and 41 from other areas of China. Our study is focused on (i) genetic background of potato cultivars in China, (ii) contribution to potato disease resistance breeding.

MATERIAL AND METHODS

Plant materials. Seventy-six elite potato cultivars (Table S1 in electronic supplementary material (ESM)) collected from fifteen provinces of China were used in this study. Among them, 35 cultivars were collected from southwest (Chongqing, Guizhou, Sichuan, Xizang) and the other 41 cultivars were collected from other parts of China. All cultivars were grown in the greenhouse of Tuber and Crop Research Institute (TCRI), Yunnan Agricultural University.

DNA isolation, PCR and electrophoresis. For each cultivar, young expanding leaves from 3 random seedlings were collected, bulked, immediately frozen in liquid nitrogen and stored at -80°C . Genomic DNA was isolated by the modified CTAB procedure (MOISAN-THIERY *et al.* 2005). DNA quality was examined using 0.8% agarose gel.

Seven PCR-based DNA markers were used to identify disease resistance, including four for PLB resistance gene (*R1*, *R2*, *R3a*, and *R3b*), two for PVY resistance gene (*Ry_{chc}* and *Ry_{adg}*) and one for PVX (*Rx1*) resistance gene. Primer sequences, optimized concentration and annealing temperature for each primer pair are shown in Table S2 in ESM. PCR reactions were conducted in a 10- μl volume consisting of 2 μl (50–100 ng/ μl) of template DNA, 1.0 μl of 10 \times PCR buffer, 0.6 μl of MgCl_2 (25 mM), 0.8 μl of dNTP Mixture (2.5 mM), 0.25 units Taq DNA

polymerase (TIANGEN BIOTECH, Beijing, P.R. China) and the corresponding primers. The thermal cycling procedure was one cycle of 10 min at 94°C , followed by 35 cycles of 30 s at 94°C , 30 s at different annealing temperatures (Table S2 in ESM), and 1 min at 72°C for elongation (except for 1.5 min for *R1* marker), followed by final extension with one cycle of 5 min at 72°C . All the reactions were performed on a TProfessional thermal cycler (Biometra, Göttingen, Germany). PCR products were separated by electrophoresis on a 1.4% agarose gel. All PCRs were repeated at least twice to verify the veracity.

For genetic diversity analysis, based on previously described SSR markers and evaluation in our laboratory, twelve highly informative SSR markers were used in this study (GHISLAIN *et al.* 2004; FEINGOLD *et al.* 2005; REID & KERR 2007; LIAO & GUO 2014). Primer information is shown in Table S3 in ESM. The PCR reaction system and thermal cycling procedure were used according to LIAO and GUO (2014). The polyacrylamide electrophoresis was used according to BENBOUZA *et al.* (2006) with a small improvement: 6 μl of these denatured products were loaded and electrophoresis was run for about 1 hour at 1200 V, then the products were visualized by silver staining. The acquired allelic data were scored as allele size for calculating the allelic features as well as the 0 (absence) and 1 (presence) matrix for preparing and comparing a dendrogram. The Jaccard similarity coefficient was used to evaluate genetic similarities between each pair of lines by using the NTSYS-pc 2.10 software package (Biostatistics Inc., Oro Valley, USA). Cluster analysis (SAHN clustering) based on the UPGMA (Unweighted Pair Group Method with Arithmetic Average) method was performed for preparing the dendrogram.

RESULTS AND DISCUSSION

Disease resistance evaluation based on late blight and potato virus disease resistance linked markers.

A total of 76 potato cultivars were screened for the presences of PLB (*R1*, *R2*, *R3a*, *R3b*), PVY (*Ry_{adg}*, *Ry_{chc}*) and PVX (*Rx1*) disease resistances (Figure 1, 2, and Table 1). As for PLB resistance, the results showed a high percentage of resistance in the tested cultivars with 57.89 and 32.89% for the *R3a* and the *R3b* loci, respectively. However, only 6 (Yushu 2, Zhengshu 5, Lishu 1, Aihuashuimo, Kangqing 901 and TP262) out of the 76 (7.9%) cultivars tested contained more than two late blight resistance markers. Among them,

all four PLB markers can be successfully identified in TP262. This cultivar has a great potential as the late blight resistant source for potato breeding. As for virus disease resistance, only 3 accessions (Yunshu 103, Lishu 7 and Eshu 5) amplified marker *Rx1*. As a result, potential resistance to PVX might be relatively rare in the 76 tested accessions. More accessions amplified the marker associated with PVY resistance at the *Ry_{adg}* locus (22.37%) than at the *Ry_{chc}* locus (7.89%). Furthermore, there were only 3 cultivars (Wuhua potato, Unica and Chunshu 5) containing PVY resistance at both the *Ry_{chc}* and *Ry_{adg}* locus. These results suggested that these 3 accessions may be the effective PVY resistance resources.

Additionally, among the above-mentioned 6 accessions (which have more than two late blight resistance markers), Aihuashuimo is the only germplasm resource containing a virus resistance marker (*Ry_{adg}*-linked marker). It illustrated that the accessions with

late blight resistance markers lacked PVY or PVX resistance markers. In fact, the accessions containing both late blight and PVY resistance markers represent a 15.8% ratio in all 76 tested accessions. Therefore, it is significant to pyramid late blight and virus disease resistance genes in the tested accessions. Except two PVY resistance markers, Wuhua potato showed *R3a* and *R3b* related markers, reflecting an application potential in pyramiding late blight and virus resistance genes. The marker *Rx1* was present in 3 accessions, Yunshu 103, Lishu 7 and Eshu 5, among them Eshu 5 lacked all but *Rx1*-linked marker. Besides the marker *Rx1*, Lishu 7 amplified the marker *R3a*. Only Yunshu 103 contained markers of PVX, PVY and PLB resistance genes. Seven out of 76 (9.2%) accessions lacked all markers analysed in this study, however, potential accessions such as Aihuashuimo, TP262 and Yunshu 103 useful for resistance gene pyramiding were found. As published by OTTOMAN *et al.*

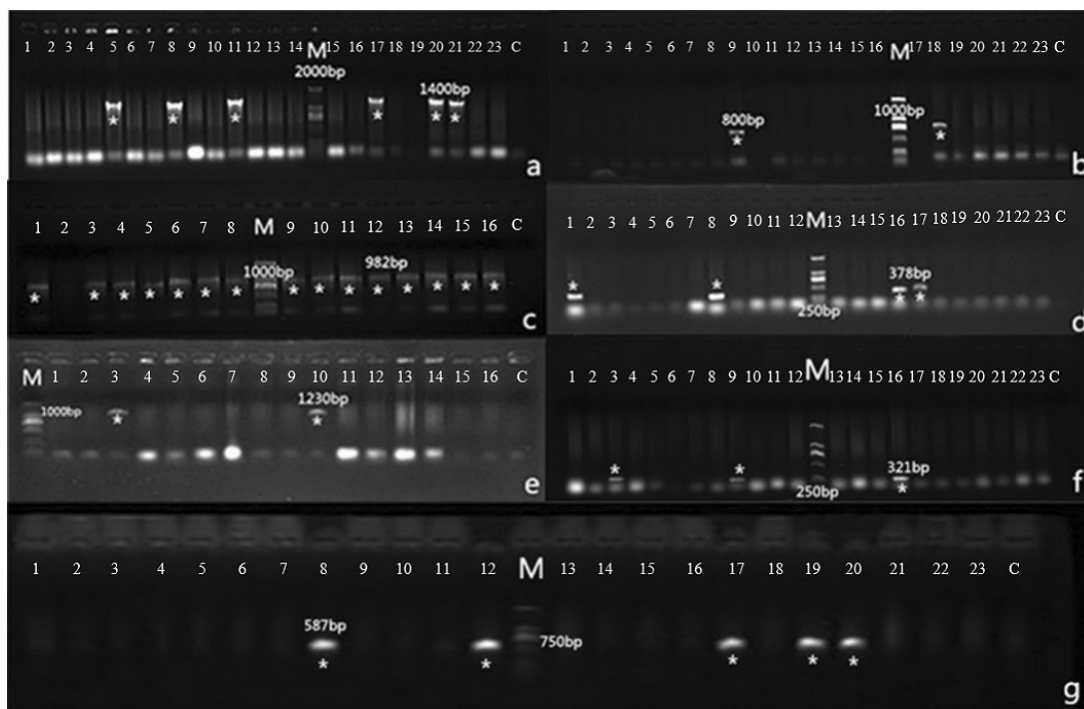


Figure 1. Detection of the resistance genes *RI* (a), *R2* (b), *R3a* (c), *R3b* (d), *Rx1* (e), *Ry_{adg}* (f) and *Ry_{chc}* (g) in analysed accessions using corresponding diagnostic DNA markers

Marker fragments scored positively are indicated by *; M – DNA size maker, 2000-bp ladder; C – the negative control without template DNA; 1 – Hui-2; 2 – Changguohong; 3 – Yunshu 103 (except in panel (d), which is Emma); 4 – Zhuangshu 3; 5 – Zhengshu 5 (except in panel (d), which is Anshu 1); 6 – Dingshu 1; 7 – Mian potato; 8 – Lishu 1 (except in panel (g), which is Wuhua potato); 9 – Aihuashuimo (except in panel (a), which is Anshu 1); 10 – Lishu 7; 11 – Zaodabai; 12 – Yanshu 4 (except in panel (a), which is Emma); 13 – Shixuan 11; 14 – Ed 53; 15 – Zhongdianhong; 16 – White potato; 17 – Lishu 6 (except in panel (g), which is Unica); 18 – Longshu 3 (except in panel (a), which is Mira); 19 – Chunshu 5 (except in panel (g), which is Emma); 20 – Longshu 5; 21 – Zhongshu 5; 22 – Xuanshu 4; 23 – Zhongshu 20

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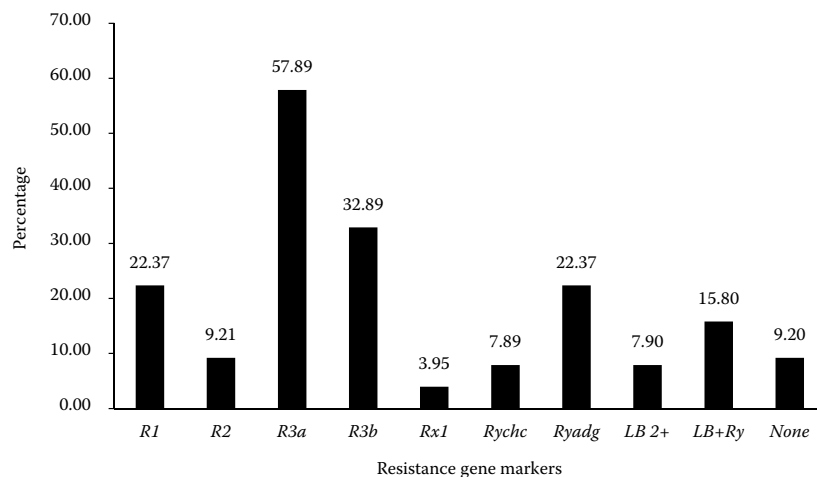


Figure 2. The percentage of accessions having corresponding marker bands

LB2+ means the percentage of accessions which had more than two late blight resistant gene marker bands in this study; LB+Ry means the percentage of accessions in which both diagnostic DNA markers of late blight and PVY were detected; none means the percentage of accessions which lacked all marker bands

(2009), through generating and testing populations with a large number of individuals, the chances of generating a resistant potato variety will be greatly increased. However, the accurate phenotype testing of advanced clones can be started only after the population has been reduced to a manageable size (TAI & YOUNG 1984). Therefore, in order to test large populations in limited time and with limited cost, MAS is an absolutely efficient method.

Because of abundant mountainous areas and plateaus, the southwestern area showed prominent three-dimensional climate. In this area, potatoes could be harvested once or twice with different cultivation patterns. Also, in China, about 40% of potatoes are produced in the southwest (JANSKY *et al.* 2009). Therefore, 35 accessions (46.1%) from this area were collected as a part of detecting materials. It is noteworthy that more typical accessions mentioned above came from the southwest of China. For example, among materials which have more than two late blight resistance markers, 4 out of 6 were collected from the southwest of China, including TP262 containing four markers of late blight resistance. Although only three accessions contained the marker *Rx1*, two of them (Yunshu 103 and Lishu 7) originated from the southwest of China. Also, the results indicated that the southwestern accession Wuhua potato (with markers *Ry_{hc}*, *Ry_{adg}*, *R3a* and *R3b*) and Yunshu 103 (with markers *Rx1*, *Ry_{adg}*, *R3a* and *R3b*) reflected a promising application potential in pyramiding the late blight and virus disease resistance. The reason

might be the special climate in the southwest of China. In most parts of this area, there is no chilly winter and hot summer (XIONG *et al.* 2012). Cloud cover and frequent mists could provide a suitable environment for late blight pathogens (JANSKY *et al.* 2009). In addition, this warm weather makes aphids more vibrant. After potato harvest, some other plants, such as oilseed rape, could be the new hosts of aphids (HOOKS & FERERES 2006). As a result, the late blight and virus diseases are more severe in the southwestern area and breeders here have to pay more attention to resistance characteristics. Because of this kind of selection, the southwestern area might contain more typical materials.

Molecular genetic diversity evaluation. SSR loci were used to evaluate the genetic diversity of 76 elite potato accessions in China. The dendrogram (Figure 3, Table S1 in ESM) showed that most of the cultivars (except No.13 – Netherlands 15 and No.17 – Luyin 1) were discriminated by the 12 SSR markers. There was no clear clustering of accessions by planting area observed in the dendrograms. Two major groups and four minor groups were observed. Two major groups (Group I and Group III) account for 60 accessions (78.95%) and 11 accessions (14.47%), respectively. Cooperation 88 and Mira were clustered within Group IV, while Dingshu 1, Ed 53 and Zhongdianhong were clustered within different groups separately (Group II, V, VI).

The genetic diversity study showed that the genetic similarity coefficient between accessions ranged from 0.4839 to 0.8266 with an average of 0.6568. In

Table 1. Detection of diagnostic DNA markers of late blight and virus disease resistance genes in potato accessions

Code	Accession	R1	R2	R3a	R3b	Rx1	Ry _{chc}	Ry _{adg}	Code	Accession	R1	R2	R3a	R3b	Rx1	Ry _{chc}	Ry _{adg}
1	Zhongshu 5	+	-	-	-	-	-	-	39	Jingbian 1	-	-	-	+	-	-	-
2	Tianshu 11	-	-	+	-	-	-	+	40	Favorita	-	-	-	+	-	-	-
3	Longshu 6	-	+	+	-	-	-	-	41	Jinshu 16	-	-	+	-	-	-	-
4	Tianshu 10	-	-	+	+	-	-	+	42	Yushu 1	-	-	+	+	-	-	-
5	Longshu 3	+	+	-	-	-	-	-	43	Mira	-	-	-	-	-	-	-
6	Dingshu 1	-	-	+	-	-	-	-	44	Anshu 1	-	-	+	-	-	-	-
7	Xindaping	+	-	-	-	-	-	-	45	Liangshu 14	-	-	+	+	-	-	-
8	Longshu 5	+	-	-	-	-	+	-	46	Changguohong	-	-	-	-	-	-	-
9	Zhuangshu 3	-	-	+	-	-	-	-	47	Emma	-	-	-	-	-	-	-
10	Jizhangshu 8	-	-	-	-	-	-	+	48	Shizong 1	-	-	-	-	-	-	+
11	Jizhangshu 12	-	-	-	-	-	-	-	49	White potato	-	-	+	+	-	-	+
12	Kexin 2	-	-	+	+	-	-	-	50	Shizong potato	-	-	-	+	-	-	-
13	Netherlands 15	-	-	+	-	-	-	-	51	3810	-	-	-	-	-	-	+
14	Kexin 13	-	-	+	+	-	-	-	52	Hui-2	-	-	+	+	-	-	-
15	Kexin 18	+	-	-	+	-	-	-	53	Cooperation 88	+	+	-	-	-	-	-
16	Kexin 1	-	-	+	+	-	-	-	54	Lishu 7	-	-	+	-	+	-	-
17	Luyin 1	-	-	-	-	-	-	-	55	Dinong 1	-	-	-	-	-	-	+
18	Zhongshu 7	-	-	+	-	-	-	-	56	Xiaowuyu	-	-	-	-	-	-	-
19	Dongnong 303	-	-	-	-	-	-	+	57	Xuanshu 4	-	-	-	-	-	-	-
20	Zhongshu 20	-	-	-	-	-	-	-	58	Mian potato	-	-	+	-	-	-	-
21	Kexin 21	-	-	+	-	-	-	-	59	Tiechanghong potato	+	-	+	-	-	-	-
22	Kexin 6	-	-	+	+	-	-	+	60	γ-2	-	-	-	-	-	-	+
23	Yushu 2	+	+	+	-	-	-	-	61	Weiyu 3	-	-	+	+	-	-	-
24	Zhengshu 5	+	-	+	+	-	-	-	62	Shixuan 11	-	-	+	-	-	-	-
25	Zhengshu 6	-	-	+	-	-	-	-	63	Wuhua potato	-	-	+	+	-	+	+
26	Luo potato 8	-	-	+	-	-	-	-	64	Big eye potato	-	-	+	+	-	-	-
27	Nuanzhoujin 8	-	-	-	-	-	-	-	65	Lishu 1	+	-	+	+	-	-	-
28	Eshu 5	-	-	-	-	+	-	-	66	Kangqing 9-1	+	-	+	+	-	-	-
29	Xinyu 4	-	-	+	+	-	-	-	67	Ed 53	-	-	+	-	-	-	-
30	Russian 7	-	-	-	+	-	-	-	68	Yunshu103	-	-	+	+	+	-	+
31	Chunshu 5	-	-	-	-	-	+	+	69	Yunshu 301	-	-	+	-	-	-	+
32	Shepody	-	-	-	-	-	-	-	70	Yunshu 201	-	-	-	-	-	-	+
33	Yanshu 4	+	-	+	-	-	+	-	71	TP262	+	+	+	+	-	-	-
34	Zaodabai	+	-	+	-	-	-	-	72	Lishu 6	+	-	-	+	-	-	-
35	Ningshu 4	-	-	+	-	-	-	-	73	Aihuashuimo	+	+	+	-	-	-	+
36	Unica	-	+	-	-	-	+	+	74	JS03-136	-	-	+	-	-	-	+
37	Qingshu168	-	-	+	-	-	-	-	75	Ludianshuimo	+	-	-	-	-	-	-
38	Zihuabai	-	-	+	+	-	-	-	76	Zhongdianhong	-	-	+	-	-	-	-

summary, few accessions showed to be the genetically diverse genotypes. Potato breeding in China is a relatively new venture. It begins after the earliest foreign cultivars were introduced in the 1940s (JANSKY *et al.* 2009). Ninety-three cultivars released

before 1983 were shown to have a narrow genetic background due to the common parentage (MIN 2008). Our results revealed that the genetic backgrounds of Chinese potatoes with five groups and similarity ranging from 0.4839 to 0.8266 were still

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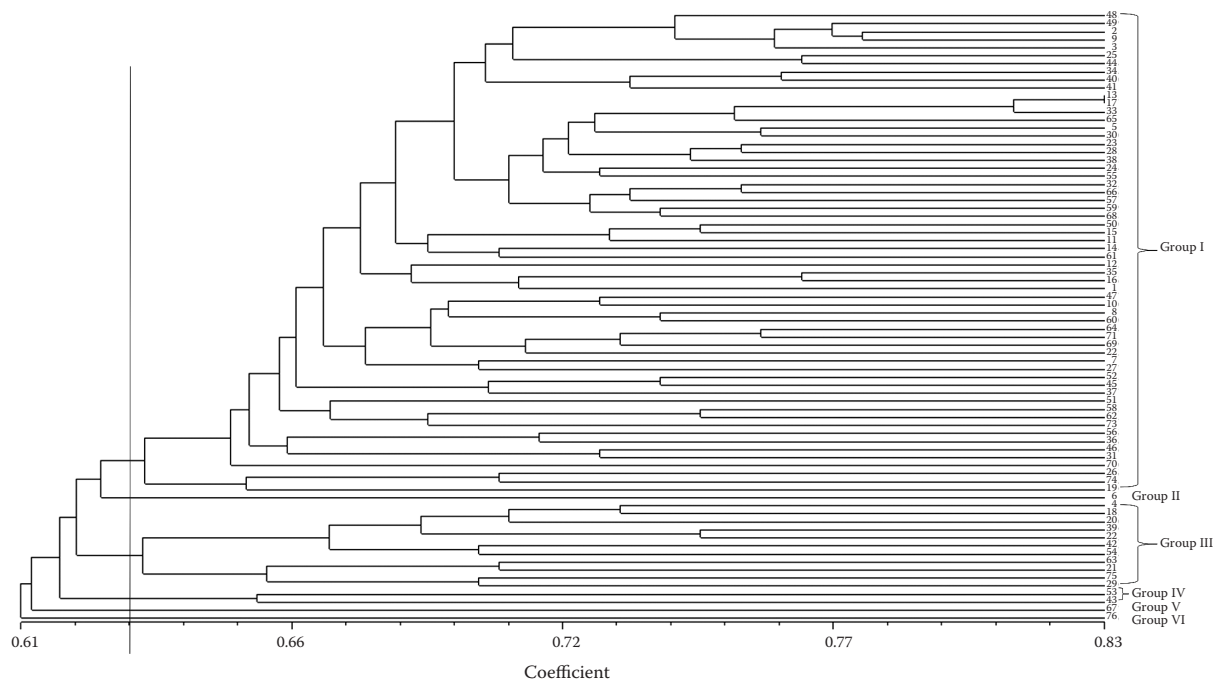


Figure 3. Dendrogram of 76 elite potato accessions in China; values on the X-axis correspond to coefficients of similarity; numbers in brackets indicate the code of accessions which are the same as Table 1

narrow even in recent years. This was consistent with the previous reports on the genetic diversity analysis of 88 approved potato cultivars (DUAN *et al.* 2009). Therefore, utilization of wild species or primitive cultivars adapted locally and introduction of new germplasm are significant for expanding the genetic diversity of Chinese potatoes. Although a relatively narrow genetic diversity was observed in the present study, the materials which were clustered separately from main groups may have some application values in parent selection. Utilization of these cultivars and multiple resistant materials, such as TP262, Wuhua potato and Yunshu 103, may provide great opportunities for improving disease resistance in potato cultivars of China. SSRs and quantitative estimate of genetic diversity enable excluding genotypes with close genetic relationships from designated crosses.

Comparing genetic parameters and southwestern conditions, the genetic similarity coefficient in the southwest was a little lower. The average genetic similarity coefficient among southwestern accessions was 0.6508 and ranged from 0.4839 to 0.7775. From Figure 4, it was clear that southwestern materials had genetic similarity coefficients which were lower than 0.60. It indicated that the genetic diversity level in southwestern materials was higher than the average genetic diversity level. Specially, five accessions

clustered separately from main groups may have some application values in parent selection. And four of them were collected from the southwest of China. Nowadays, the cross between Cooperation 88 (cultivated in the southwest and clustered separately, with markers *R1*, *R2*) and Yunshu103 (clustered in Group I, with markers *Rx1*, *Ry_{adg}*, *R3a* and *R3b*)

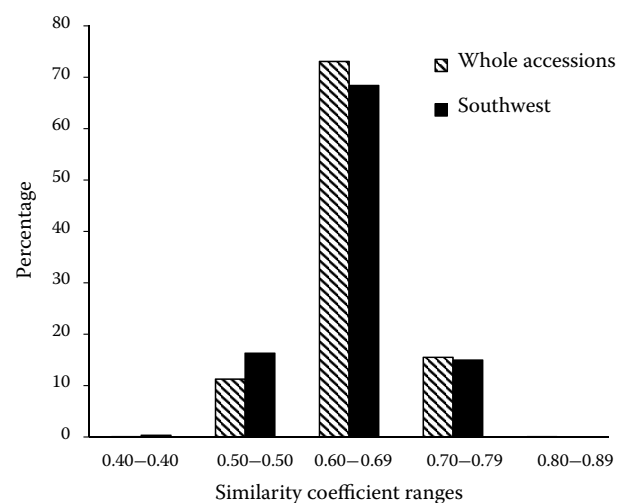


Figure 4. Frequency distribution of pairwise simple sequence repeat (SSR) similarity coefficients among all accessions and 35 southwestern accessions

has been developed in order to find more valuable genotypes for disease resistance breeding.

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