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Genetic Diversity of 18 Bramble Cultivars Introduced Abroad and 8 Wild Materials of *Rubus coreanus* from China Revealed by RAPD

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Abstract: In this study, the genetic diversity and relationship of 18 bramble cultivars and 8 materials from *Rubus coreanus* were analyzed by RAPD markers. Of the 85 random primers screened, 20 primers gave reproducible and polymorphic bands. Total 251 bands were generated by using 20 RAPD primers, of which 235 (93.6%) were polymorphic. The average number of bands generated per primer was 12.5 with a range from 5-25. The results showed that the genetic distance between Coreanus 3 and 4, both collected in Xichong County, Sichuan Province, was the nearest, while the genetic distance between yellow raspberry 'Kiwigold' and blackberry 'Olallie' was the farthest. The dendrogram of cluster analysis showed that raspberry cultivars and *R. coreanus* had close genetic relationship, since they clustered into a group. Black raspberry 'Bristol' and blackberry cultivars clustered into another group. The genetic relationships and diversity within the cultivars or materials from *R. coreanus* was complex, for they didn't cluster together according to their fruit color or original place.

Key words: *Rubus*, *R. coreanus*, bramble cultivars, genetic diversity, RAPD

INTRODUCTION

The genus *Rubus* is one of the largest genera in the *Rosaceae*, consisting of 750~1000 species in many parts of the world (Lu, 1983; Thompson, 1997). Those *Rubus* plants used for horticultural plantations are generally named bramble or edible *Rubus* (Gui and Hu, 2002). Due to its nutritional values and medical uses, *Rubus* fruits were named fruits of life by Americans and brambles were recommended as the third generation fruits by FAO (Xu *et al*., 2003). So far, brambles have been widely cultivated in many parts of the world and have increasingly become the fourth rapidly developing small fruit next to strawberries, blueberries and currants (Weber, 2006). Appropriate cultivars is the key to develop planting, which are decided by the quantity and quality of genetic resources owned and the depth and width studied, so is brambles industry. China is one of the original centers of bramble, which has more than 200 species of wild materials (Gu *et al*., 1993), but most cultivars of bramble in China were introduced abroad, which had led to many problems such as ecological inadaptation, cultivars confusion and using restriction (Wang *et al*., 2006). The very imminent thing needing to do is to breed its own bramble cultivars. A detailed knowledge and understanding of the genetic relationship among cultivars and local wild materials is essential for the success of plant breeding programs as well as for efficient sampling and more informed utilization of available germplasm (Finn *et al*., 2006).

DNA-based molecular marker techniques such as random polymorphic DNA (RAPD) (Williams *et al*., 1990) have been provided to be powerful tools. Since, the RAPD technique did not require any previous knowledge of the target genome and is relatively simple and rapid to carry out, RAPD markers have been extensively used in population genetics, analyses of biodiversity and studies of relationships among species at different levels (Williams *et al*., 1993). Some studies using RAPD markers in *Rubus* have been reported. Graham and McNicol (1995) found that RAPD data could be used to identify cultivars, species and interspecific crosses. Stafne *et al*. (2003) differentiated several blackberry and raspberry cultivars for genetic identification by pedigree and RAPD analyses and found that the RAPD data were more reliable than pedigree. In the case of Chinese local wild materials, there were few papers that had reported the application of the RAPD marker technique to identify the genetic diversity between introduced cultivars and local wild materials. The aims of this study were to screen decamer oligonucleotide primers with relevance in bulk analysis, to determine genetic diversity and relationships using RAPD

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markers among 18 bramble cultivars introduced abroad and 8 wide materials of species of *Rubus coreanus* collected in China.

MATERIALS AND METHODS

Plant materials: In total, 26 bramble genotypes including 18 cultivars introduced from 5 countries and 8 wide materials of *Rubus coreanus* collected in China were used in this study (Table 1). Plant materials were grown in the teaching and researching garden in Sichuan Agricultural University. Young leaves were collected and stored at -70°C until used.

Genomic DNA extraction: Total DNA was extracted from the stored leaves according to the protocol described by Lodhi (1994). The RNA contamination in all the samples was removed by digesting the extract with 10 μg of RNase-A for 30 min at 37°C. DNA quality was tested using 1% agarose gel electrophoresis and its concentration was determined spectrophotometrically. The plant DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 4°C for instant use and -20°C for longer storage.

RAPD amplication and gel eletrophoresis: The PCR reaction was carried out in a 25 μL volume of a mixture containing 2.5 μL 10×PCR Buffer, 2.0 mM $MgCl_2$, 0.25 mM dNTPs, 0.3 μM primer, 1.5 unit Taq DNA polymerase and 20 mg DNA template. Amplification was performed in a programmable Mastercycler Gradient PCR Engine (Eppendorf) programmed for an initial denaturation at 94°C for 4 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 36°C for1 min and extension at 72°C for 2 min, with a final extension at 72°C for 10 min. PCR products were electrophoresed in a 1.5% agarose gel in 0.5×TBE (Tris-Borade-EDTA) buffer at 100 V for 2 h and stained with ethidium bromide (0.5 μg mL^{-1}). DNA fragments were then visualized by illumination with UV light. Amplification was repeated at least twice for each sample and only the reproducible fragments were scored for final data matrix.

Data analysis: Photographs from ethidium bromide stained agarose gels were used to score RAPD data for analysis. The presence of the product was scored as 1 and absence as 0. Data was subjected to matrix and cluster analysis using the numerical taxonomy and multivariate analysis system program package for PC (NTSYS-pc version 2.1) (Rohlf, 2000). Percentages of polymorphic bands were defined as the percentage of polymorphic bands amplified by a single primer to that of the total number of bands produced by the same primer. To evaluate genetic diversity at population level (Nei, 1972) standard genetic distances between all pairs of populations were calculated based on frequencies of RAPD bands in individual population. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) using Sequential Agglomerative, Hierarchical and Nested Cluster (SAHN) (Sneath and Sokal, 1973).

Table 1: Materials used in this study

Code	Name	Taxon	Original place	Parents
1	Algonquin	Summer-bearing red raspberry	Canada	Haida×Canby
2	Chilcotin	Summer-bearing red raspberry	Canada	Sumner×Newburgh
3	Chilliwack	Summer-bearing red raspberry	Canada	Skeena x (Sumner×Carnival)
4	Killarney	Summer-bearing red raspberry	Canada	Chief×Indian Summer
5	Reveille	Summer-bearing red raspberry	American	(Indian Summer×Sunrise)×September
6	Tulameen	Summer-bearing red raspberry	Canada	Nootka×Glen Prosen
7	Dinkum	Fall-bearing red raspberry	Australia	Autumn Bliss×Glen Moy
8	Nova	Fall-bearing red raspberry	Canada	Southland×Boyne
9	Polana	Fall-bearing red raspberry	Poland	Heritage×Zeva Herbsternte
10	Kiwigold	Yellow raspberry	New Zealand	sport of Heritage
11	Bristol	Black raspberry	America	Watson Prolific×Honeysweet
12	Black Butte	Thorn-trailing blackberry	America	ORUS 830-4×ORUS 728-3
13	Boysen	Thorn-trailing blackberry	America	Unknown
14	Navaho	Thornless-erect blackberry	America	(Thornfree×Brazos)×(Ark-550×Cherokee)
15	Olallie	Thorn-trailing blackberry	America	Black Logan×Young
16	Shawnee	Thorn-erect blackberry	America	Cherokee×(Thornfree×Brazos)
17	Kotata	Thorn-trailing blackberry	America	(Pacific×Boysen)×(Jenner-1×Eldorado)
18	Arapaho	Thornless-erect blackberry	America	(Ark-550×Cherokee)×Ark-883
19	Coreanus 1	*R. coreanus*	Xichong, China	Wild
20	Coreanus 2	*R. coreanus*	Xichong, China	Wild
21	Coreanus 3	*R. coreanus*	Xichong, China	Wild
22	Coreanus 4	*R. coreanus*	Xichong, China	Wild
23	Coreanus 5	*R. coreanus*	Xichong, China	Wild
24	Coreanus 6	*R. coreanus*	Ya'an, China	Wild
25	Coreanus 7	*R. coreanus*	Ya'an, China	Wild
26	Coreanus 8	*R. coreanus*	Ya'an, China	Wild

RESULTS

Polymorphism analysis: Eight five RAPD primers were initially screened to amplify the genomic DNA of three samples ('Algonquin', Black Butte' and Coreanus 1). Twenty primers that gave clear and polymorphic bands were used in subsequent experiments. For 26 materials listed in Table 1, the 20 RAPD primers amplified a total of 251 bands, of which 235 bands (93.6%) were polymorphic (Table 2). The average number of DNA bands amplified by each primer was 12.5 with a range from 5-25. The primer 6 yielded the largest number of bands (25), while primer 2 amplified the fewest number of bands (5). The size of amplified bands ranged from approximately 200-4000 bp. The RAPD patterns of 26 materials amplified by primer 9 were presented in Fig. 1.

Genetic distance analysis: Using the RAPD within population band frequencies (Nei, 1972) standard genetic distances could be estimated between all pairs of bramble populations (Table 3). The genetic distance ranged from 0.065-0.730 with an average of 0.410. The highest genetic distance (0.730) was observed between 'Kiwigold' (yellow raspberry) and 'Olallie' (blackberry) and the lowest value (0.065) was between Coreanus 3 and Coreanus 4. Among 11 raspberry cultivars, 'Bristol' and 'Polana' had the highest genetic distance (0.579), while 'Algonquin' and 'Killarney' had the lowest value (0.089). Among 7 blackberry cultivars 'Shawnee' and 'Arapaho' had closet genetic relationship (0.135); Black Butte' and 'Navaho' had the farthest (0.327). *R coreanus* had the genetic distance value ranging from 0.065-0.335.

Cluster analysis: The UPGMA dendrogram based on genetic distance for 26 materials was given in Fig. 2. Twenty-six materials were grouped into two large clades, A and B. All the *R coreanus* and all but one cultivar of raspberry were grouped in Clade A. The only exception was 'Bristol', which was grouped in Clade B. Clade B contained all cultivars of blackberry.

Clade A was further divided into two subclades, A1 and A2. Subclade A1 was composed of 10 cultivars. The red raspberry cultivars 'Algonquin', 'Killarney', 'Chilcotin' and 'Chilliwack' which all originated from Canada were firstly grouped. The other cultivars were not grouped according to their original place or fruit color. Subclade A2 was composed of all the materials of *R coreanus*. Coreanus 3 and 4, both collected in Xichong county had the closest genetic relationship. Clade B was also divided into two subclades, B1 and B2. There was only one material the black raspberry 'Bristol' in Subclade B1, while the blackberry cultivars all originated from America in Subclade B2.

Table 2: Primers selected for amplification and polymorphism revealed

Primer	Sequence (5'→3')	Total bands	Polymorphic bands	Polymorphism rate (%)
1	TCACGTCCAC	14.0	14.0	100.0
2	CAGGGGTGGA	5.0	4.0	80.0
3	GTGATCGCAG	15.0	15.0	100.0
4	TCGGCGATAG	21.0	21.0	100.0
5	TTCCGAACCC	14.0	13.0	92.9
6	AGCCAGACGA	25.0	25.0	100.0
7	GTGCCTAACC	7.0	7.0	100.0
8	CTGACGTCAC	12.0	9.0	75.0
9	AATCGGGCTG	20.0	20.0	100.0
10	GGCACTGAGG	11.0	8.0	72.7
11	GAGCCCTCCA	8.0	7.0	87.5
12	GTCAGGGCAA	10.0	10.0	100.0
13	GAAACGGGTG	11.0	11.0	100.0
14	GGTAGCAGTC	9.0	8.0	88.9
15	GGTCCTCAGG	6.0	5.0	83.3
16	ACTCAGGAGC	10.0	9.0	90.0
17	CCACCGCCAG	13.0	11.0	84.6
18	AGAGTCGCCC	12.0	11.0	91.7
19	CAGTTCGAGG	11.0	11.0	100.0
20	AGCCAGCGAA	17.0	16.0	94.1
Total		251.0	235.0	-
Average		12.6	11.8	-
Polymorphism		-	-	93.6

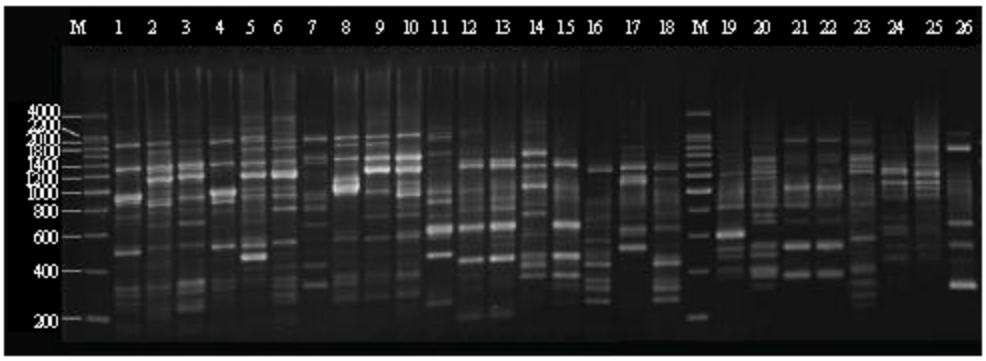

Fig. 1: DNA amplification profile of eighteen bramble cultivars and 8 wild materials of *R coreanus* with RAPD primer 9. The lane numbers are same as the sample numbers in Table 1. M: 200 bp DNA marker

Table 3: Genetic distances matrix among the 26 bramble populations based on RAPD data

1	2	3	4	5	6	7	8	9	10	11	12	13
0.000												
0.151	0.000											
0.149	0.082	0.000										
0.089	0.143	0.142	0.000									
0.236	0.155	0.206	0.189	0.000								
0.215	0.189	0.204	0.253	0.182	0.000							
0.224	0.207	0.223	0.253	0.191	0.122	0.000						
0.166	0.185	0.183	0.193	0.179	0.203	0.186	0.000					
0.231	0.212	0.238	0.221	0.223	0.203	0.230	0.147	0.000				
0.208	0.199	0.225	0.217	0.184	0.245	0.273	0.144	0.143	0.000			
0.516	0.520	0.509	0.548	0.472	0.451	0.451	0.566	0.579	0.562	0.000		
0.531	0.582	0.571	0.597	0.561	0.463	0.463	0.630	0.595	0.627	0.271	0.000	
0.521	0.572	0.593	0.619	0.505	0.512	0.512	0.570	0.521	0.583	0.400	0.177	0.000
0.498	0.517	0.521	0.605	0.543	0.426	0.426	0.518	0.514	0.558	0.344	0.327	0.276
0.540	0.645	0.616	0.626	0.638	0.499	0.530	0.607	0.606	0.730	0.457	0.189	0.167
0.522	0.557	0.577	0.585	0.553	0.473	0.445	0.512	0.538	0.599	0.369	0.307	0.284
0.564	0.583	0.604	0.596	0.562	0.438	0.438	0.597	0.579	0.643	0.347	0.243	0.277
0.527	0.546	0.566	0.591	0.542	0.476	0.476	0.516	0.527	0.605	0.455	0.326	0.245
0.355	0.352	0.409	0.413	0.397	0.328	0.349	0.378	0.405	0.420	0.470	0.500	0.474
0.380	0.353	0.400	0.380	0.364	0.382	0.406	0.426	0.457	0.434	0.531	0.515	0.553
0.393	0.400	0.462	0.405	0.423	0.407	0.442	0.427	0.495	0.435	0.629	0.649	0.618
0.380	0.399	0.449	0.380	0.398	0.394	0.429	0.414	0.470	0.422	0.599	0.582	0.571
0.348	0.367	0.367	0.396	0.402	0.307	0.307	0.370	0.411	0.414	0.474	0.458	0.495
0.372	0.391	0.443	0.436	0.440	0.385	0.409	0.431	0.478	0.479	0.646	0.573	0.509
0.345	0.364	0.412	0.404	0.398	0.316	0.337	0.390	0.384	0.410	0.531	0.498	0.472
0.400	0.395	0.471	0.463	0.382	0.344	0.355	0.363	0.403	0.352	0.543	0.596	0.532

14	15	16	17	18	19	20	21	22	23	24	25	26
0.000												
0.304	0.000											
0.166	0.255	0.000										
0.287	0.323	0.266	0.000									
0.192	0.273	0.135	0.228	0.000								
0.454	0.525	0.434	0.470	0.452	0.000							
0.525	0.613	0.504	0.515	0.494	0.190	0.000						
0.653	0.723	0.598	0.647	0.658	0.254	0.275	0.000					
0.623	0.671	0.569	0.617	0.627	0.231	0.230	0.065	0.000				
0.427	0.533	0.435	0.459	0.470	0.243	0.243	0.335	0.311	0.000			
0.579	0.587	0.576	0.627	0.583	0.262	0.275	0.208	0.185	0.220	0.000		
0.479	0.542	0.474	0.482	0.494	0.221	0.187	0.160	0.137	0.221	0.112	0.000	
0.475	0.609	0.485	0.511	0.521	0.227	0.215	0.238	0.247	0.249	0.271	0.183	0.000

The numbers of 1~26 correspond to the materials codes listed in Table 1

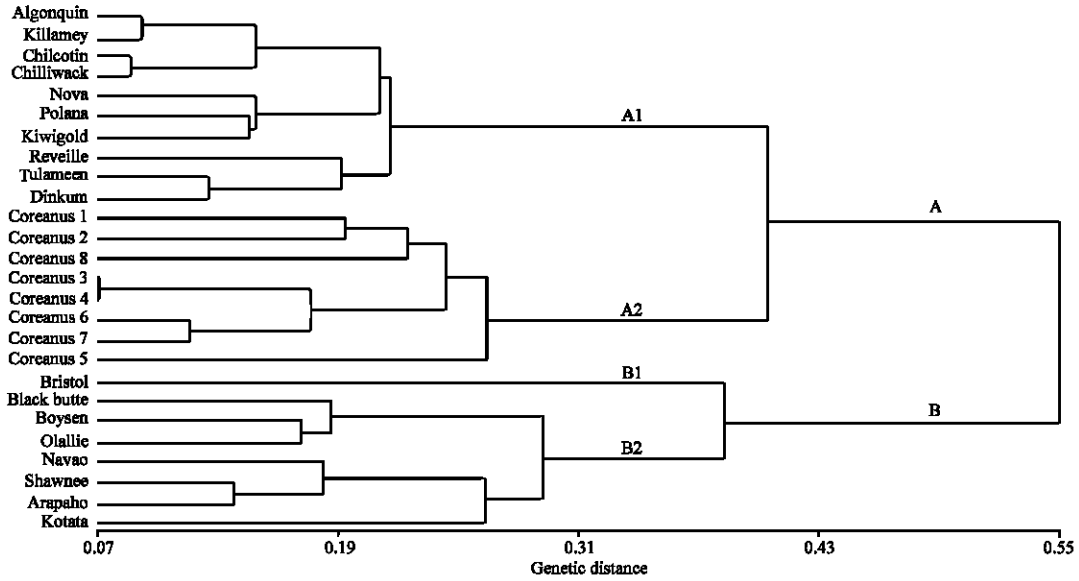

Fig. 2: Dendrogram of 26 bramble populations based on genetic distance

DISCUSSION

Genetic relationship among raspberry, *R. coreanus* and blackberry: Of the bramble varieties, the most popular ones were the red raspberry (*R. idaeus*) and the blackberry (*R. laciniatus*). Hybrids between the red and black raspberry were commonly called purple raspberries because of the fruit and cane color. However, most taxonomists did not recognize hybrids as distinct species. Interspecific hybrids with blackberries had also been made, such as Loganberry, Boysenberry and Youngberry (Pritts and Handley, 1989). Raspberry fruit consisted of an aggregate of drupelets that pull away from the floral receptacle, which were hollow. While blackberry drupelets adhere to the receptacle, which were solid. In the case of *R. coreanus*, their fruits were hollow. So raspberry and *R. coreanus* had close relationship according to their fruit characteristics. The results of cluster analysis in this study, coincided with the above in molecular level. Otherwise, black raspberry fell into the same clade withblackberry although it contained a hollow core. This result was consistent with previous results obtained from pollen morphology for wild species and cultivars of brambles (Wang *et al*., 2007).

Genetic diversity within populations: The color of raspberry fruit was very abundant. They could come in black, shades of purple and in various shades of yellow, from off-white to gold. Raspberries could also be divided into summer-bearing raspberry and fall-bearing raspberry according to their fruiting habit (Weber, 2006). In this study, we used summer-bearing red raspberry, fall-bearing red raspberry, yellow raspberry and black raspberry as materials. The results showed that they didn't grouped by their fruit color or fruiting habit. For blackberry cultivars, 'Boysen', 'Olallie' and 'Black Butte' gathered together firstly with the characteristics of thorny and trailing. 'Shawnee', 'Arapaho' and 'Navaho' which were thorn and erect fell into the other group. Arapaho' and 'Navaho' were very important germplasm for the thornless stems. As the Chinese local materials and excellent germplasm resources, *R. coreanus* had big potentialities for breeding towards fruit quality, productivity, resistance and adaptation (Li *et al*., 2002). In this study, different original wild materials of *R. coreanus* with different number of leaflets showed abundant genetic diversity through RAPD marker method.

CONCLUSION

We conclude that the genetic diversity of the introduced bramble cultivars and *R.coreanus* from China showed: High rate of polymorphic bands (93.6%) were amplified by using twenty RAPD primers; *R. coreanus* and raspberry cultivars had closer genetic relationship; black raspberry and blackberry had closer genetic relationship; the genetic relationships and diversity within the populations was complex, since they didn't cluster together according to their fruit color or original place.

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