# Genetic Relationships among 17 Bramble Cultivars and 11 Wild Excellent *Rubus* Germplasms from China Revealed by RAPD

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**Abstract:** Genetic relationships among 17 bramble cultivars introduced abroad and 11 wild excellent *Rubus* germplasms from China were analyzed by RAPD markers. A total of 500 DNA bands were amplified by 22 primers and 490 out of 500 bands (98%) were polymorphic. The average number of polymorphic DNA bands amplified by each primer was 22.28. All materials could be distinguished by RAPD markers with 0.687-0.964 genetic similarity coefficients. According to the data, a dendrogram of genetic relationship, which was constructed using the UPGMA method, showed that all the tested cultivars and wild species (varieties) were classified into four groups. The present results verify that the close genetic relationship between ten raspberry cultivars and seven excellent wild germplasms from section (subgenus) *Idaeobatus* of the genus *Rubus*, while a little far genetic relationship between ten raspberry cultivars and seven blackberry cultivars. There was a far genetic relationship between seven wild germplasms from section (subgenus) *Idaeobatus* and four wild germplasms from section (subgenus) *Malachobatus*. The two cultivated bramble groups (raspberry and blackberry) could be completely distinguished, but it was failed to distinguish red raspberry with even red raspberry in raspberry group.

**Key words:** Genetic relationship, bramble, germplasm, *Rubus*, RAPD, cluster analysis

## INTRODUCTION

Brambles (*Rubus* L.) included raspberry, blackberry, dewberry and other *Rubus* plants with utilization potential. In China, wild brambles, as fruits purpose, were exceptionally rich, up to >30 species (Wang *et al.*, 2006), some of which like *R. biflorus*, *R. ellipticus*, *R. coreanus*, *R. niveus* and *R. parvifolius*, due to some excellent traits such as productivity, fruit quality, adaptation and resistance had been used in breeding programs and cultivar development by many breeders from China and abroad (Gu *et al.*, 1989; Thompson, 1995; Li *et al.*, 2002). However, only four bramble cultivars had been obtained in China since, the 2005 year (Zheng *et al.*, 2000; Wu *et al.*, 2002, 2005; Liu *et al.*, 2005), the limited knowledges of the genetic data in the excellent wild bramble germplasms from China as well as the genetic relationships between them and commercial brambles cultivars abroad were probably important reason.

In order to accelerate bramble breeding process in China, it very necessary to obtain molecular data on these wild excellent brambles to breeding utilization as well as the genetic relationships among them and those excellent commercial bramble cultivars abroad. In the present study, the genetic relationships among the eleven wild excellent bramble germplasms, which widely distributed in the southwest of China and the seventeen excellent bramble cultivars introduced abroad would be explored by RAPD markers.

## MATERIALS AND METHODS

Twenty eight materials were analyzed in this study (Table 1). Total genomic DNA was isolated from frozen young leaves using the procedure of nuclear DNA protocols (Zhou, 2005).

A total of 22 polymorphic primers selected from 85 RAPD primers were used for *Rubus* plants. The RAPD components, which followed the earlier study, that was, in a total of 25 µL volume, which contained 1×PCR buffer, 2.0 mM $MgCl_2$, 0.24 mM dNTPs, 0.6 µM primer, 20 ng template DNA and 1.5 unit of Taq DNA polymerase. PCR amplification was performed in a PTC-200 cycler (MJ Research). PCR conditions were 45 cycles at 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and a final extension at 72°C for 10 min. The PCR products were separated on 1.5% agarose gel (with 0.5 µg $mL^{-1}$ EtBr) using 1×TBE buffer. DNA fragments were visualized and photographed by Bio-RAD

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Table 1: The samples used in this study and their locality (or ancestres)

| Samples | Collection place/source | Accession/ancestry | Number |
|---|---|---|---|
| **Excellent wild bramble germplasms** | | | |
| **Sect. *Idaeobatus* Focke** | | | |
| *R. niveus* | Yaan, Sichuan | R01-1 | 1 |
| *R. parvifolius* | Xichong, Sichuan | R03-23 | 2 |
| *R. ellipticus* var. *obcordatus* | Yaan, Sichuan | R01-2 | 3 |
| *R. coreanus* | Yaan, Sichuan | R01-4 | 4 |
| *R. hirsutus* | Longquan, Sichuan | R07-1 | 5 |
| *R. eustephanus* | Kangding, Sichuan | R09-1 | 6 |
| *R. corchorifolius* | Yaan, Sichuan | R01-6 | 7 |
| **Sect. *Malachobatus* Focke** | | | |
| *R. lambertianus* var. *glaber* | Yaan, Sichuan | R01-8 | 25 |
| *R. multibracteatus* | ShiBing, Guizhou | R20-1 | 26 |
| *R. setchuenensis* | Tiqnquan, Sichuan | R05-3 | 27 |
| *R. buergeri* | Yaan, Sichuan | R01-11 | 28 |
| **Cultivars** | | | |
| **Red raspberry** | | | |
| Chilliwack | Canada | - | 9 |
| Algonquin[a] | Canada | Haida x Canby | 10 |
| Chilcotin | Canada | - | 11 |
| Reveille[a] | America | India summer x Sunrise | 8 |
| Killarney | - | - | 14 |
| **Even red raspberry** | | | |
| Dinkum | Australia | - | 12 |
| Nova[a] | Canada | Southland x Boyne | 13 |
| Ploana[b] | Poland | - | 15 |
| **Yellow raspberry** | | | |
| Kiwigold | - | - | 16 |
| **Black raspberry** | | | |
| Bristol | America | - | 17 |
| **Blackberry** | | | |
| Shawnee[c,d] | America | Cherokee x AR.586 (Thornfree x Brazos) | 18 |
| Navaho[c,d] | America | ARK583(Thornfree x Brazos) x ARK631(ARK550 x Cherokee) | 19 |
| Arapaho[d] | America | ARK631(ARK550 x Cherokee) x ARK883(ARK593 x ARK650) | 20 |
| Ollalie[c,d] | America | Black Logan x Young | 24 |
| Black butte | America | - | 21 |
| Kotata[c,d] | - | OSC743 (Pacific x Boysen) x OSC877(Jenner-1 x Eldorado) | 22 |
| Boysen[d] | New Zealand | *R. ursinus* | 23 |

*The ancestry of cultivars refered to a): Wang *et al.* (2003); b): Weber (2007); c): Meng and Finn (1999) and d): Thompson (1995)

Gel 2000 gel scanner. The RAPD results were recorded as 0 (missing) and 1 (present) data matrix and analyzed by NTSYS-PC software (version 2.10). During construct the dendrograms, the similarity coefficients and the UPGMA (unweighted pairgroup method with arithmetic average) and the SAHN (sequential, hierarchical, agglomerative and nested clustering) methods were adopted.

## RESULTS AND DISCUSSION

A total of 500 bands were produced by 22 primers and 490 out of 500 bands (98%) were polymorphic. Twelve to thirteen polymorphic bands were amplified by each primer, with an average of 22.28 (Table 2). Twenty eight materials could be distinguished from each other by 22 primers in the study. The RAPD result in 28 materials produced by primer 1204-218 was shown in Fig. 1. The genetic similarity coefficients ranged from 0.687-0.964. The similarity coefficients were used to generate a dendrogram with UPGMA (Fig. 2). From the dendrogram, the 28 materials could be divided into four groups.

In group 1, seven taxa *R. niveus*, *R. coreanus*, *R. hirsutus*, *R. eustephanus*, *R. corchorifolius*, *R. parvifolius* and *R. ellipticus* var. *obcordatus* from Sect. *Idaeobatus* are clustered together and the average of genetic similarity coefficient among them was 0.860.

In group 2, ten raspberry cultivars, contained five red raspberry Reveille, Chilliwack, Algonquin, Chilcotin and Killarney, three even red raspberry Dinkum, Nova and Ploana, one yellow raspberry Kiwigold and one black raspberry Bristol were grouped together. The average of genetic similarity coefficient among them was 0.903.

In group 3, seven blackberry cultivars were clustered together, Shawnee, Navaho, Arapaho, Ollalie, Black Butte, Kotata and Boysen. The average of genetic similarity coefficient among them was 0.921.

In group 4, four taxa *R. lambertianus* var. *glaber*, *R. multibracteatus*, *R. setchuenensis* and *R. buergeri* from Sect. *Malachobatus* were grouped together and the average of genetic similarity coefficient among them was 0.862.

Table 2: The sequence of the primers producing polymorphic bands and their amplified results

| Order number | Sequence | No. of amplified bands | No. of polymorphic bands | Percentage of polymorphic bands |
|---|---|---|---|---|
| 1204-209 | TCACGTCCAC | 18 | 18 | 100 |
| 1204-211 | CAGGGGTGGA | 12 | 12 | 100 |
| 1024-212 | GTGACGTAGG | 20 | 19 | 95 |
| 1204-213 | GTGATCGCAG | 22 | 22 | 100 |
| 1204-214 | TCGGCGATAG | 27 | 27 | 100 |
| 1204-215 | TTCCGAACCC | 19 | 19 | 100 |
| 1204-216 | AGCCAGACGA | 22 | 22 | 100 |
| 1204-217 | GGTGATCAGG | 22 | 21 | 95.45 |
| 1204-218 | GTGCCTAACC | 33 | 33 | 100 |
| 1204-219 | CTGACGTCAC | 25 | 25 | 10 |
| 1204-220 | AATCGGGCTG | 28 | 28 | 100 |
| 1204-221 | GGCACTGAGG | 22 | 22 | 100 |
| 1204-222 | GAGCCCTCCA | 16 | 15 | 93.75 |
| 1204-223 | GTCAGGGCAA | 25 | 24 | 96 |
| 1204-225 | GGTAGCAGTC | 25 | 25 | 100 |
| 1204-226 | GGTCCTCAGG | 25 | 24 | 96 |
| 1204-227 | TCGGAGTGGC | 18 | 18 | 100 |
| 1204-228 | ACTCAGGAGC | 17 | 13 | 76.47 |
| 1204-230 | AGAGTCGCCC | 30 | 30 | 100 |
| 1204-231 | CAGTTCGAGG | 26 | 26 | 100 |
| 1204-232 | AGCCAGCGAA | 17 | 16 | 94.12 |
| 1204-233 | AGCCAGACGA | 31 | 31 | 100 |
| Total | - | 500 | 490 | - |
| Average | - | 22.73 | 2.28 | 98 |
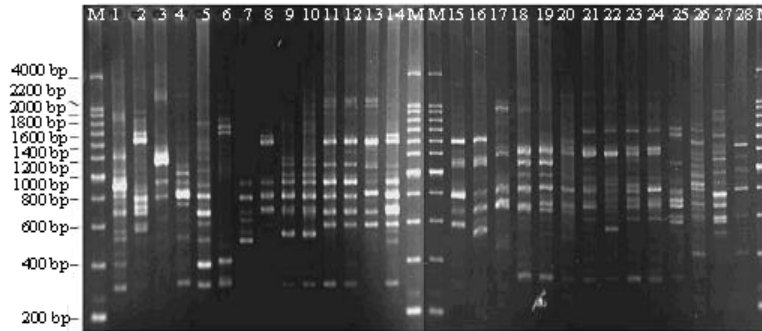


Fig. 1: Results of RAPD polymorphism in bramble wild resource and cultivars simplified by marker 1204-218. Lanes 1-28: 1): *R. niveus*, 2): *R. parvifolius*, 3): *Ra. ellipticus* var. *obcordatus*, 4): *R. coreanus*, 5): *R. hirsutus*, 6): *R. eustephanus*, 7): *R. corchorifolius*, 8): Reveille, 9): Chilliwack, 10): Algonquin, 11): Chilcotin, 12): Dinkum, 13): Nova, 14): Killarney, 15): Ploana, 16): Kiwigold, 17): Bristol, 18): Shawnee, 19): Navaho, 20): Arapaho, 21): Black Butte, 22): Kotata, 23): Boysen, 24): Ollalie, 25): *R. lambertianus* var. *glaber*, 26): *R. multibracteatus*, 27): *R. setchuenensis* and 28): *R. buergeri* and M): DNA maker

The average of genetic similarity coefficient of group 2 and 3 showed that quite close relationship among cultivars in the same cultivated population introduced in this study and indicated that the genetic diversity of these cultivars was narrow, although there were a lot of germplasms used to breeding programs of bramble abroad (Thompson, 1995; Hummer, 1996; Hummer and Finn, 1999; Stafne and Clark, 2004).

Raspberry cultivars were selected from those germplasms taxonomical belonging to section (subgenus) *Idaeobatus* of the genus *Rubus* and blackberry cultivars were selected from those germplasms taxonomical belonging to section (subgenus) *Rubus* of the genus *Rubus* (Gu, 1992; Thompson, 1995). The present results also verify that the close genetic relationship between ten raspberry cultivars and seven excellent wild germplasms from section (subgenus) *Idaeobatus* of the genus *Rubus*, while a little far genetic relationship between ten raspberry cultivars and seven blackberry cultivars, with respective genetic similarity coefficient of 0.830 and 0.812. The genetic similarity coefficient of group I and group IV was 0.756 and there was a far genetic relationship between seven wild germplasms from section (subgenus) *Idaeobatus* and four wild germplasms from section (subgenus) *Malachobatus*.

The two cultivated groups could be completely reflected, but it was failed to distinguish red raspberry with even red raspberry. For example, there were genetic

Table 3: Genetic similarity matrix among 17 bramble cultivars and 11 wild *Rubus* germplasms from China based on polymorphic RAPD bands*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 0.834 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 0.831 | 0.894 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 0.855 | 0.835 | 0.838 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | | |
| 0.845 | 0.833 | 0.830 | 0.848 | 1.000 | | | | | | | | | | | | | | | | | | | | | | | |
| 0.860 | 0.837 | 0.848 | 0.866 | 0.918 | 1.000 | | | | | | | | | | | | | | | | | | | | | | |
| 0.882 | 0.856 | 0.871 | 0.874 | 0.896 | 0.914 | 1.000 | | | | | | | | | | | | | | | | | | | | | |
| 0.851 | 0.825 | 0.844 | 0.845 | 0.842 | 0.867 | 0.895 | 1.000 | | | | | | | | | | | | | | | | | | | | |
| 0.829 | 0.821 | 0.830 | 0.815 | 0.822 | 0.850 | 0.873 | 0.939 | 1.000 | | | | | | | | | | | | | | | | | | | |
| 0.820 | 0.814 | 0.823 | 0.814 | 0.819 | 0.843 | 0.864 | 0.931 | 0.964 | 1.000 | | | | | | | | | | | | | | | | | | |
| 0.824 | 0.820 | 0.819 | 0.822 | 0.827 | 0.849 | 0.872 | 0.915 | 0.921 | 0.930 | 1.000 | | | | | | | | | | | | | | | | | |
| 0.815 | 0.815 | 0.811 | 0.813 | 0.818 | 0.842 | 0.861 | 0.898 | 0.904 | 0.911 | 0.968 | 1.000 | | | | | | | | | | | | | | | | |
| 0.810 | 0.808 | 0.809 | 0.807 | 0.814 | 0.836 | 0.850 | 0.902 | 0.889 | 0.894 | 0.923 | 0.928 | 1.000 | | | | | | | | | | | | | | | |
| 0.816 | 0.813 | 0.819 | 0.806 | 0.819 | 0.845 | 0.860 | 0.899 | 0.890 | 0.887 | 0.910 | 0.919 | 0.946 | 1.000 | | | | | | | | | | | | | | |
| 0.813 | 0.809 | 0.808 | 0.803 | 0.812 | 0.837 | 0.856 | 0.897 | 0.884 | 0.885 | 0.912 | 0.917 | 0.935 | 0.945 | 1.000 | | | | | | | | | | | | | |
| 0.821 | 0.810 | 0.811 | 0.807 | 0.822 | 0.844 | 0.863 | 0.891 | 0.879 | 0.876 | 0.897 | 0.902 | 0.904 | 0.925 | 0.942 | 1.000 | | | | | | | | | | | | |
| 0.836 | 0.821 | 0.824 | 0.831 | 0.816 | 0.840 | 0.865 | 0.869 | 0.865 | 0.854 | 0.860 | 0.857 | 0.861 | 0.866 | 0.869 | 0.878 | 1.000 | | | | | | | | | | | |
| 0.803 | 0.781 | 0.778 | 0.783 | 0.792 | 0.815 | 0.839 | 0.808 | 0.782 | 0.773 | 0.771 | 0.763 | 0.759 | 0.770 | 0.764 | 0.774 | 0.802 | 1.000 | | | | | | | | | | |
| 0.803 | 0.779 | 0.778 | 0.781 | 0.790 | 0.813 | 0.839 | 0.804 | 0.782 | 0.773 | 0.770 | 0.761 | 0.757 | 0.768 | 0.762 | 0.772 | 0.802 | 0.957 | 1.000 | | | | | | | | | |
| 0.822 | 0.799 | 0.798 | 0.801 | 0.810 | 0.833 | 0.858 | 0.825 | 0.800 | 0.791 | 0.789 | 0.780 | 0.776 | 0.787 | 0.781 | 0.792 | 0.821 | 0.947 | 0.947 | 1.000 | | | | | | | | |
| 0.821 | 0.797 | 0.796 | 0.803 | 0.812 | 0.836 | 0.861 | 0.824 | 0.800 | 0.792 | 0.792 | 0.783 | 0.779 | 0.790 | 0.784 | 0.793 | 0.820 | 0.900 | 0.904 | 0.924 | 1.000 | | | | | | | |
| 0.822 | 0.799 | 0.798 | 0.803 | 0.812 | 0.835 | 0.858 | 0.825 | 0.802 | 0.793 | 0.791 | 0.782 | 0.778 | 0.789 | 0.783 | 0.794 | 0.819 | 0.900 | 0.898 | 0.916 | 0.939 | 1.000 | | | | | | |
| 0.828 | 0.807 | 0.806 | 0.808 | 0.817 | 0.841 | 0.862 | 0.831 | 0.808 | 0.799 | 0.797 | 0.788 | 0.784 | 0.795 | 0.789 | 0.800 | 0.825 | 0.904 | 0.900 | 0.916 | 0.939 | 0.957 | 1.000 | | | | | |
| 0.818 | 0.797 | 0.794 | 0.797 | 0.806 | 0.829 | 0.851 | 0.819 | 0.796 | 0.787 | 0.785 | 0.776 | 0.772 | 0.783 | 0.777 | 0.788 | 0.811 | 0.895 | 0.893 | 0.906 | 0.922 | 0.938 | 0.938 | 1.000 | | | | |
| 0.798 | 0.780 | 0.777 | 0.778 | 0.789 | 0.813 | 0.830 | 0.805 | 0.779 | 0.771 | 0.769 | 0.760 | 0.756 | 0.767 | 0.761 | 0.770 | 0.785 | 0.792 | 0.792 | 0.812 | 0.810 | 0.813 | 0.817 | 0.808 | 1.000 | | | |
| 0.751 | 0.731 | 0.730 | 0.729 | 0.742 | 0.764 | 0.781 | 0.756 | 0.730 | 0.722 | 0.720 | 0.711 | 0.707 | 0.718 | 0.712 | 0.719 | 0.738 | 0.745 | 0.745 | 0.763 | 0.761 | 0.765 | 0.769 | 0.761 | 0.873 | 1.000 | | |
| 0.734 | 0.713 | 0.710 | 0.713 | 0.726 | 0.743 | 0.765 | 0.733 | 0.710 | 0.701 | 0.699 | 0.690 | 0.687 | 0.697 | 0.691 | 0.700 | 0.719 | 0.723 | 0.727 | 0.742 | 0.743 | 0.746 | 0.750 | 0.740 | 0.845 | 0.878 | 1.000 | |
| 0.756 | 0.734 | 0.735 | 0.740 | 0.749 | 0.771 | 0.790 | 0.759 | 0.733 | 0.725 | 0.725 | 0.716 | 0.712 | 0.723 | 0.717 | 0.724 | 0.743 | 0.750 | 0.752 | 0.770 | 0.770 | 0.771 | 0.775 | 0.766 | 0.841 | 0.874 | 0.859 | 1.000 |

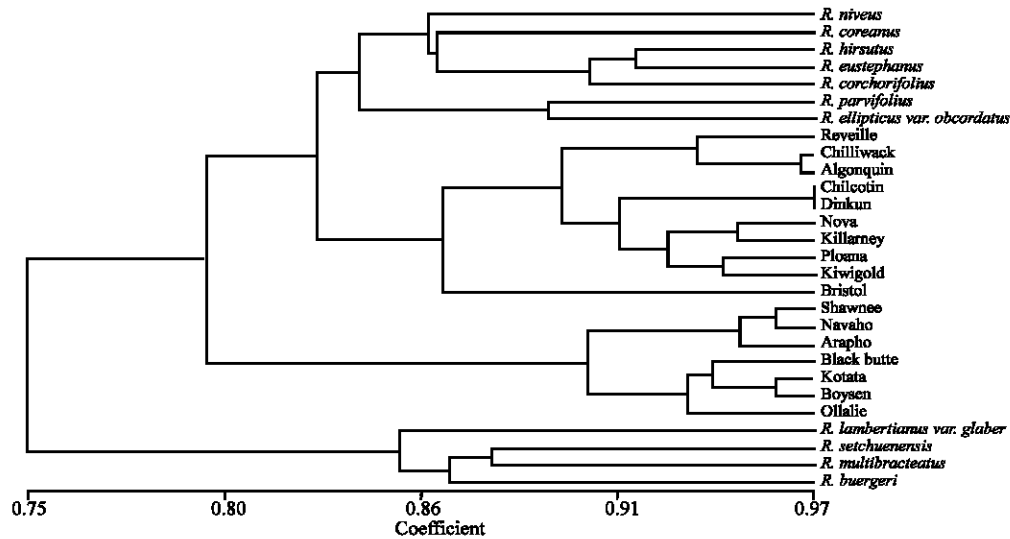The material order from 1-28 is described in the same order as in Table 1



Fig. 2: A dendrogram generated from RAPD markers for 11 *Rubus* bramble wild resource and 17 cultivars

relationship between red raspberry Chicotin and even red raspberry Dinkum were closer than any one of raspberry cultivated populations and genetic similarity coefficient was 0.968 and the genetic relationship between Killarney and Nova was close too, with genetic similarity coefficient was 0.946. In addition, there was a chose genetic relationship between yellow raspberry Kiwigold and even red raspberry Ploana and genetic similarity coefficient was 0.942 (Table 3).

## CONCLUSION

Eleven wild excellent *Rubus* germplasms from China and 17 bramble cultivars could be distinguished by RAPD makers and divided into four groups. There was a far genetic relationship between 11 wild excellent *Rubus* germplasms from China and any one of two cultivated populations introduced abroad. The RAPD technique was an effective additional method for assessing the genetic relationships of *Rubus*.

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