Genetic Relationship of Ornamental Peach Determined Using AFLP Markers

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Abstract. Ornamental peach (Prunus persica (L.) Batsch) is a popular plant for urban landscapes and gardens. However, the genetic relationship among ornamental peach cultivars is unclear. In this report, a group of 51 ornamental peach taxa, originated from P. persica and P. davidiana (Carr.) Franch., has been studied using AFLPs. The samples were collected from China, Japan, and US. A total of 275 useful markers ranging in size from 75 to 500 base pairs were generated using six EcoRI/MseI AFLP primer pairs. Among them, 265 bands were polymorphic. Total markers for each taxon ranged from 90 to 140 with an average of 120. Two clades were apparent on the PAUP–UPGMA tree with P. davidiana forming an outgroup to P. persica, indicates that P. davidiana contributed less to the ornamental peach gene pools. Within P. persica clade, 18 out of 20 upright ornamental peach cultivars formed a clade, which indicated that cultivars with upright growth habit had close genetic relationship. Five dwarf cultivars were grouped to one clade, supported by 81% bootstrap value, indicating that they probably derived from a common gene pool. These results demonstrated that AFLP markers are powerful for determining genetic relationships in ornamental peach. The genetic relationships among ornamental cultivars established in this study could be useful in ornamental peach identification, conservation, and breeding.

Prunus persica (L.) Batsch (ornamental peach) is a small deciduous tree of Rose Family (Rosaceae). The plant has glabrous branchlets, serrulate leaves, and subsessile flowers blooming before the unfolded leaves during the early spring. The showy flowers range from pink to red, white, bicolor or tricolor. Growth habits range from upright, dwarf, weeping, to fastigiate. Leaf colors vary from green to purple (Hu et al., 2003). All these characteristics make the ornamental peach an excellent plant for urban landscapes and gardens.

Ornamental peach had been cultivated in China for thousands of years before it was introduced to western countries about three hundred years ago (Everett, 1967). Today it is grown in Asia, Europe, North America, South Africa, and Australia for its outstanding ornamental features and many new cultivars have been introduced in the nursery trades. However, ornamental peach nomenclature and classification in the literature are contradictory and confusing. Some ornamental peach cultivars have the same name in the literature, but do not have the similar morphological descriptions (Krußmann, 1986; Moore et al., 1993). Others have different names, but share identical morphological characteristics (Jacobson, 1996). It is difficult to classify ornamental peach taxa based only on morphological characteristics (Dirr, 1998). Modern technology can aid in clarifying nomenclature, and in determining genetic relationships.

AFLP procedure. Amplified fragment length polymorphism restriction, ligation, and preselective amplification reactions were conducted according to the Perkin Elmer AFLP Plant Mapping Protocol (PE Applied Biosystems, Foster City, Calif.) and following the manufacturer’s protocols. DNA concentration was quantified using an Eppendorf BioPhotometer (Brinkmann Instruments, Inc., Westbury, N.Y.). All DNA samples were diluted or concentrated to a uniform concentration of 80 µg/mL for the following AFLP procedures.

AFLP data analysis. For each primer combination, the amplified fragments were analyzed using GeneScan software (version 3.1. Perkin Elmer, Applied Biosystems) equipped with ABI 377. The presence and absence of a specific relationships for agronomic crops, fruit trees and ornamental plants, such as Cephalotaxus (Zhang et al., 2000), Dahlia (Debener, 2002), daylily (Tomkins et al., 2001), Dendranthema (Zhou and Dai, 2002), Dieffenbachia (Chen et al., 2004), Lagemstroemia (Pooler, 2003), Philodendron (Devanand et al., 2004), plum (Goulao et al., 2001), Prunus mume (Ming and Zhang, 2003), and sweet cherry (Struss et al., 2001, 2003; Tavaud et al., 2001; Zhou et al., 2002).

Previous studies of fruiting peach cultivars (Aranzana et al., 2001, 2003; Dirlewanger et al., 1998, 2001, 1999; Shimada et al., 1998) have shown that AFLP technique produces a high degree of polymorphic markers per assay, which could provide an efficient system for detection and analysis of fruiting peach cultivars.

In this study, therefore, AFLP was applied to estimate genetic relationships of ornamental peach taxa, providing insights into their classification and further breeding.

Materials and Methods

Plant materials. Fifty-one ornamental peach taxa, which originated from P. persica and P. davidiana, were used in this study. They were collected from the following botanical gardens and arboretas: Beijing Botanical Garden, Beijing, China (BBG), National Institute of Fruit Tree Science, Tsukuba, Japan (JAPAN), and the JC Raulston Arboretum at North Carolina State University, Raleigh, North Carolina, USA (ICRA) (Table 1). The relationships among these cultivars were described by Hu et al. (2003) based on their morphological features.

DNA extraction. Total genomic DNA was isolated from silica gel dried leaves using a DNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, Calif.) and following the manufacturer’s protocols. DNA concentration was quantified using an Eppendorf BioPhotometer (Brinkmann Instruments, Inc., Westbury, N.Y.). All DNA samples were diluted or concentrated to a uniform concentration of 80 µg/mL for the following AFLP procedures.

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Results and Discussion

In total, 275 markers, whose sizes ranged from 75 to 500 base pairs (bp), were generated using six EcoRI/MseI AFLP primer combinations of 51 ornamental peach taxa. Among them, 256 markers were polymorphic. The number of markers for each taxon ranged from 90 to 140 (mean = 120, Table 2).

Based on 275 useful AFLP markers, genetic distances among the cultivars were found to be ranged from 0.044 to 0.404 (data not presented). The greatest distance (0.404) was between P. davidiana 'BBG1' and P. persica 'Clarisse', 'Zhu Fen Chui Zhi', 'Genpaishidare', and 'Corinthian White', respectively. This result was not surprising given that genetic distances among the cultivars derived from different species are expected to be higher than those among the cultivars selected within a species.

Two major clades were recognized in the UPGMA tree, one being accessions of P. davidiana (CD), and the other containing cultivars of P. persica (CP) (Fig. 1). Prunus davidiana formed an outgroup to P. persica, which indicates that P. davidiana is genetically distant from other ornamental peach taxa derived from P. persica. The clade davidiana (CD) had three taxa derived from P. davidiana (P. davidiana var. alba, P. davidiana var. rubra, and P. davidiana 'BBG1') and supported by 100% bootstrap value. The genetic distance within these three taxa is 0.16, while the average distance of these three taxa to all other taxa originated from P. persica is 0.339. Obviously, these three taxa are closely related to each other compared with ornamental peach taxa derived from P. persica. Prunus davidiana 'BBG1', a new cultivar selected by Beijing Botanical Garden (manuscript in preparation), has upright twisted branches, single pink flowers, and a more narrow growth habit than the standard

Table 1. Taxa used in this study and their parentage and key characteristics.

| Taxon | Name | Parentage | Key characteristics |
|-------|------|-----------|---------------------|
| 1 | Prunus persica 'Terutemomo' | (Houki Momo × 'Akashidara') F1 | Fastigiate, pink flower, double |
| 2 | 'Corinthian Mauve' | One seedling selected from 'NC174RL' × Pillar 271 | Fastigiate, rose pink flower, double |
| 3 | 'Teruteshiro' | (Houki Momo × 'Sansetsu Shidare') F2 | Fastigiate, white flower, double |
| 4 | 'Corinthian White' | One seedling selected from 'NC174RL' × Pillar 64 | Fastigiate, white flower, double |
| 5 | 'Corinthian Rose' | One seedling selected from 'NC174RL' × Pillar 248 | Fastigiate, rose pink flower, red-leaved |
| 6 | 'Corinthian Pink' | One seedling selected from 'NC174RL' × Pillar 172 | Fastigiate, pink flower, double |
| 7 | 'Houki Momo' | Unknown | Pink and white flower, double |
| 8 | 'Shiroshidare' | 'Akashidara' OP seedlings selection | Weeping, white flower, single |
| 9 | 'Lv E Chui Zhi' | Unknown | Weeping, white flower, double |
| 10 | 'Akashidare' | Unknown | Weeping, red flower, single |
| 11 | 'Hong Yu Chui Zhi' | Unknown | Weeping, red flower, double |
| 12 | 'Clarisse' | Unknown | Weeping, pink flower, double |
| 13 | 'Zhu Fen Chui Zhi' | Unknown | Weeping, pink flower, double |
| 14 | 'Dai Yu Chui Zhi' | Unknown | Weeping, light pink flower, double |
| 15 | 'Genpaishidare' | Unknown | Weeping, pink and white flower, single |
| 16 | 'Yuan Yang Chui Zhi' | Unknown | Weeping, pink and white flower, double |
| 17 | 'Wu Bao Chui Zhi' | Unknown | Weeping, light pink flower |
| 18 | 'Dan Ban Shou Fen' | Unknown | Dwarf, pink flower, single |
| 19 | 'Shou Fen' | Unknown | Dwarf, pink flower, double |
| 20 | 'Liang Fen Shou Xing' | Unknown | Dwarf, bright pink flower, double |
| 21 | 'Shou Bai' | Unknown | Dwarf, white flower, double |
| 22 | 'Dan Ban Shou Hong' | Unknown | Dwarf, red flower, single |
| 23 | 'Shou Hong' | Unknown | Dwarf, red flower, double |
| 24 | 'NSCU Dwarf Double Red' | Unknown | Dwarf, pink flower, double |
| 25 | 'Xia Yu Shou Fen' | Unknown | Dwarf, and white flower, double |
| 26 | 'Red Dwarf' | ('Akame' × Juseito) F1 | Dwarf, pink flower, single, red-leaved |
| 27 | 'Bonfire Patio' | Tsukuba No.2 open-pollinated | Dwarf, pink flower, single, red-leaved |
| 28 | 'Dan Fen' | Unknown | Upright, pink flower, single |
| 29 | 'Beijing Z1' | Unknown | Upright, pink flower, single, red-leaved |
| 30 | 'Dan Hong' | Unknown | Upright, red flower, single |
| 31 | 'Dan Bai' | Unknown | Upright, white flower, single |
| 32 | 'Han Hong Tao' | Unknown | Upright, red flower, double |
| 33 | 'Er Se Tao' | Unknown | Upright, pink flower, double |
| 34 | 'Zan Fen' | Unknown | Upright, pink flower, double |
| 35 | 'Bi Tao' | Unknown | Upright, pink flower, double |
| 36 | 'Hong BI Tao' | Unknown | Upright, red flower, double |
| 37 | 'Fei Tao' | Unknown | Upright, red flower, double |
| 38 | 'Wu Bao Tao' | Unknown | Upright, pink red flower, double |
| 39 | 'Ju Tao' | Unknown | Upright, pink flower, double |
| 40 | 'Kyou Maiko' | Kikoumomo bud mutation | Upright, pink flower, double |
| 41 | 'Bai Bi Tao' | Unknown | Upright, white flower, double |
| 42 | 'Wan Bai Tao' | Unknown | Upright, white flower, double |
| 43 | 'Zi Ye Tao' | Unknown | Upright, red flower, double, red-leaved |
| 44 | 'BBG2' | Mutation selected from 'Zi Ye Tao' | Upright, red flower, bicolor leaves red and green |
| 45 | 'Unriu Tao' | Nectarine mutation | Twisted twig, pink flower, single |
| 46 | 'Bai Hua Shan Bi Tao' | Unknown | Hybrid, white flower, double |
| 47 | 'Fen Hua Shan Bi Tao' | ('He Huan Er Se Tao' × 'Bai Hua Shan Bi Tao') F2 | Hybrid, pink flower, single |
| 48 | 'Fen Hong Shan Bi Tao' | ('Jiang Tao' × 'Bai Hua Shan Bi Tao') F2 | Hybrid, rose pink flower, double |
| 49 | P. davidiana var. rubra | Unknown | Fastigiate, pink flower, single |
| 50 | P. davidiana 'BBG1' | Selected from P. davidiana var. rubra | White flower, single |
| 51 | P. davidiana var. alba | Unknown | White flower, single |

*Japan.
*JCRA.
*BBG.
upright *P. davidiana*. The genetic distance between this new cultivar and the other two *P. davidiana* varieties is 0.16 (genetic similarity is 0.84 shown on Fig. 1).

The *P. persica* clade (CP) consisted of four subgroups, clade PR (red-leaved clade), clade PT (twisted clade), clade PU (upright clade), and clade PG [growth habit clade including fastigiate, weeping, and dwarf]). Clade PR included two red-leaved cultivars (‘Zi Ye Tao’ and ‘BBG2’), with 100% bootstrap support. ‘BBG2’ is a bud-sport from the normal red-leaved ‘Zi Ye Tao’. It was selected by Beijing Botanical Garden in 2001. Compared with normal red-leaved cultivars, ‘BBG2’ has unique white and green bicolor leaves. The lowest genetic distance from this clone to ‘Zi Ye Tao’ was 0.12.

The clade PT consisted only of cultivar ‘Unriu Momo’. It is the only documented ornamental peach cultivar with twisted twigs. The plant was a mutant selected from nectarine seedlings (Yoshida et al., 2000). It is separate from all other ornamental peach taxa within the clade *P. persica* (CP) in the UPGMA tree. The average genetic distance to other *P. persica* taxa is 0.237. It is possible that this cultivar originated independently and might be an important germplasm source for further ornamental peach breeding.

Twenty taxa were clustered into the clade PU, which had the common morphological trait of upright branches. Eighteen out of 20 upright ornamental peach cultivars in this study were in this clade. Other growth habits, such as fastigiate (with narrow growth habit, columnar shape), weeping (with pendulous branches, umbrella shape), and dwarf (dense and compact, with short internodes and long narrow leaves), were mostly grouped to their relevant clade. These results demonstrate that growth habit probably is a hierarchy in ornamental peach systematics, supporting the conclusion from Hu et al. (2003).

Three hybrids, ‘Bai Hua Shan Bi Tao’, ‘Fen Hua Shan Bi Tao’, and ‘Fen Hong Shan Bi Tao’, were grouped into the clade PU. The average distance (0.192) of these three hybrids to other cultivars in the clade *P. persica* (CP) is less than that to the clade *Davidiana* (0.254), but farther from other taxa in the clade *P. persica* (CP).

The position of ‘Bai Hua Shan Bi Tao’, ‘Fen Hua Shan Bi Tao’, and ‘Fen Hong Shan Bi Tao’ in the PU clade supports the hybrid origin of these cultivars. Both ‘Fen Hua Shan Bi Tao’ and ‘Fen Hong Shan Bi Tao’ share ‘Bai Hua Shan Bi Tao’ (Hu and Zhang, 2001) as the pollen parent. The female parent of ‘Fen Hua Shan Bi Tao’ was ‘He Huang Er Se Tao’ (upright type, double, pink flowers) and the female parent of ‘Fen Hong Shan Bi Tao’ was ‘Jiang Tao’ (upright type, double, red flowers). According to the UPGMA tree, these cultivars have a closer relationship to *P. persica* than *P. davidiana*. ‘Bai Hua Shan Bi Tao’ shares 28 bands with ‘Bai Bi Tao’ and 11 bands with *P. davidiana* var. *alba*. Together, these three taxa share 61 bands in six primer combinations (Table 3). This result supports the conclusion drawn by Zhang et al. (1997) and Zhang (1998) that *P. persica* ‘Bai Hua Shan Bi Tao’ probably

| Taxon | E-ACT/M-CAT | E-AGG/M-CAT | E-ACC/M-CTC | Total |
|-------|-------------|-------------|-------------|-------|
| 1     | 24          | 20          | 11          | 108   |
| 2     | 30          | 19          | 27          | 15    |
| 3     | 28          | 22          | 23          | 17    |
| 4     | 31          | 23          | 20          | 15    |
| 5     | 26          | 23          | 29          | 18    |
| 6     | 26          | 17          | 28          | 18    |
| 7     | 25          | 22          | 32          | 19    |
| 8     | 25          | 20          | 33          | 18    |
| 9     | 25          | 19          | 25          | 15    |
| 10    | 30          | 18          | 23          | 19    |
| 11    | 31          | 19          | 25          | 17    |
| 12    | 21          | 17          | 25          | 20    |
| 13    | 27          | 18          | 27          | 18    |
| 14    | 26          | 18          | 28          | 19    |
| 15    | 31          | 17          | 25          | 17    |
| 16    | 22          | 22          | 25          | 20    |
| 17    | 23          | 21          | 23          | 18    |
| 18    | 13          | 18          | 24          | 19    |
| 19    | 8           | 18          | 25          | 14    |
| 20    | 24          | 17          | 29          | 11    |
| 21    | 7           | 18          | 20          | 19    |
| 22    | 11          | 17          | 22          | 17    |
| 23    | 9           | 18          | 21          | 19    |
| 24    | 26          | 17          | 28          | 14    |
| 25    | 22          | 16          | 26          | 19    |
| 26    | 28          | 20          | 26          | 16    |
| 27    | 30          | 21          | 32          | 19    |
| 28    | 27          | 18          | 26          | 16    |
| 29    | 29          | 27          | 27          | 19    |
| 30    | 29          | 17          | 30          | 15    |
| 31    | 30          | 16          | 20          | 9     |
| 32    | 28          | 22          | 20          | 19    |
| 33    | 26          | 21          | 22          | 20    |
| 34    | 25          | 21          | 24          | 18    |
| 35    | 30          | 23          | 22          | 19    |
| 36    | 29          | 21          | 27          | 17    |
| 37    | 24          | 21          | 24          | 19    |
| 38    | 23          | 23          | 17          | 17    |
| 39    | 34          | 21          | 19          | 21    |
| 40    | 33          | 22          | 21          | 17    |
| 41    | 29          | 23          | 26          | 18    |
| 42    | 29          | 23          | 27          | 18    |
| 43    | 35          | 27          | 21          | 18    |
| 44    | 39          | 30          | 15          | 20    |
| 45    | 36          | 28          | 30          | 18    |
| 46    | 35          | 19          | 15          | 16    |
| 47    | 33          | 18          | 21          | 19    |
| 48    | 22          | 20          | 34          | 16    |
| 49    | 23          | 25          | 16          | 14    |
| 50    | 21          | 26          | 4           | 14    |
| 51    | 23          | 28          | 17          | 11    |
| Mean  | 26          | 20          | 24          | 18    |
| Markers | 81          | 53          | 52          | 30    |

*The taxon number corresponds with the number in the first column of Table 1.*
dwarf cultivars (‘Dan Ban Shou Fen’, ‘Shou Fen’, ‘Shou Bai’, ‘Dan Ban Shou Hong’, and ‘Shou Hong’) and had 81% bootstrap support. The average distance between these five dwarf cultivars is 0.085. All of these five cultivars are very dense and compact, with short internodes, long narrow leaves. The results show that these five dwarf cultivars are more closely related to each other than to other cultivars. They probably share common germplasm.

All seven fastigiate cultivars and 10 weeping cultivars examined in this study clustered into the PGM subgroup. ‘Terutemomo’, ‘Teruteshiro’, and ‘Houki Momo’ are from Japan. The first two cultivars were released by Yamazaki et al. (1987). ‘Houki Momo’ is an ancient and unique cultivar from the Edo Era in Japan (Yoshida et al., 2000). ‘Terutemomo’ was derived from a cross of ‘Houki Momo’ and ‘Akashidare’ (clustered into the PGM as well). ‘Teruteshioro’ was derived from a cross of ‘Houki Momo’ and a weeping habit cultivar with double pink flowers, which has very similar morphological characters to ‘Zhu Fen Chui Zhi’ (also in the PGM as well). ‘Corinthian Mauve’, ‘Corinthian White’, ‘Corinthian Rose’, and ‘Corinthian Pink’ are four column-shaped cultivars released by Werner et al. (2000a, 2000b, 2000c, 2001). They are derived from ‘Houki Momo’. ‘Shiroshidare’ is an open pollinated seedling from ‘Akashidare’ (Yoshida et al., 2000). ‘Genpaishidare’ is from the National Institute of Fruit Tree Science, Tsukuba, Japan. ‘Clarisse’ is from the JC Raulston Arboretum at North Carolina State University, Raleigh, North Carolina, U.S. ‘Lv E Chui Zhi’, ‘Dai Yu Chui Zhi’, ‘Yuan Yang Chui Zhi’, ‘Hong Yu Chui Zhi’, and ‘Wu Bao Chui Zhi’ are from Beijing Botanical Garden, China. All these 17 weeping and fastigiate cultivars were clustered in one clade, suggesting that the cultivars with weeping and fastigiate growth habits have closer genetic relationships.

The cultivars with similar flower colors and flower types were scattered in different clades. ‘Kyousai’ (double red flowers) was a bud mutation from ‘Ju Tao’ (double pink flowers; Yoshida et al., 2000). The distance between these two cultivars was 0.055. Both have narrow, chrysanthemum-like petals. The distance between ‘Zan Fen’ (double pink, peony-like flowers) and ‘Fei Tao’ (double pink, peony-like flowers) is 0.076. Within the dwarf clade, the distance between ‘Shou Bai’ (double white flowers) and ‘Shou Hong’ (double red flowers) was 0.044. Within the mixed clade (PGM), the distance between ‘Hong Yu Chui Zhi’ (weeping, double red flowers) and ‘Zhu Fen Chui Zhi’ (weeping, double pink flowers) was 0.055. No distinguished cluster was formed from the
same flower color or type. This AFLP result indicates that flower color and type might not be useful in the classification of ornamental peaches.

Conclusion

AFLP fingerprinting data generated from this study provide a picture of genetic relationships of ornamental peaches. Ornamental peach taxa are mostly derived from Prunus persica. However, P. davidiana may have also been involved in ornamental peach cultivators’ breeding and development. Both the growth habit and the number of petals are important characters in the systematics of ornamental peaches. Results of this study will likely provide guidance for future germplasm collection, conservation, and breeding of ornamental peaches.

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