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Genetic characterization of different demes in *Prunus persica* revealed by RAPD markers

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Abstract

DNAs of 180 accessions in 10 demes in *Prunus persica* were amplified with twenty-two, 10-base primers selected from 200 arbitrary primers using Randomly Amplified Polymorphic DNA (RAPD) technology. One hundred and eighty loci were observed and recorded. With statistical analyses of the data from the study, genetic diversity of the demes was expressed as follow: yellow peach group > honey peach group > flat peach group > red leaf peach group > crisp peach group > bitao group and juicy peach group > nectarine group > shouxingtao group > weeping peach group. Genetic variations among and within groups by AMOVA analyses were 11.9, 88.1%, respectively. Demes clustered by UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5, the edible peaches of which were combined as a section, while the ornamental species were classified into separate sections. Through analyses of genetic diversity and genetic structure, the results could provide molecular biological evidence for conservation and utilization of *P. persica* germplasm.

Keywords: Demes of *Prunus persica*; Genetic diversity; RAPD

1. Introduction

Peach native to China has very affluent germplasm. Peach was the second major fruit in temperate zone located between southern and northern latitude from 30° to 45° through distribution of peaches in the world (James et al., 1990). The acreage and product of peach in the world were 1.41×10^6 hm^2 and 1.5405×10^7 tons (FAOSTAT Data Sources), respectively. In the procedure of peach cultivation, there were more than 5000 cultivars in the world (Association for Agricultural and Cultural, 1985) because of development of breeding and selection of peaches. Handbook of Peach and Nectarine Varieties edited by the United States Department of Agriculture (1998) recorded 800 cultivars by breeding and introduction. More than 1000 cultivars were selected in China (Wang and Zhuang, 2001). If ornamental peaches were included in the statistic, resources of peaches should be increased in large amount. Countries in the world have paid attention to collection and reservation. Nowadays, repositories were built in China, USA, French, etc. Germplasm was evaluated from agricultural and biological characters (Fruit Tree Institute, Chinese Academy of Agricultural Sciences (CAAS), 1993, 1998), which played role in conservation and use. On the other hand, above mentioned methods only provide limited information and have less capacities for discovering diversity of peaches, while molecular biological methods can disclose more information of diversity.

In the study of diversity of peaches by molecular markers, Randomly Amplified Polymorphic DNA (RAPD) (Yang et al., 2001a,b; Yuan et al., 2002; Warburton and Bliss, 1996; Badenes et al., 1998), Amplified Fragment Length Polymorphism (AFLP) (Augusto et al., 1999), Simple Sequence Repeat (SSR)(Aranzana et al., 2002; Aranzana et al., 2003; Dirlewanger et al., 2002; Wang et al., 2002), Random Amplified Microsatellite Polymorphism (RAMP)(Cheng et al., 2001) were successfully used in identification and diversity of peaches. Although diversity of peaches were studied and made some progress, there was still limitation. For instance, Warburton and Badenes preliminarily used cultivars cultivated in the USA or in Europe, respectively, while rich resources of peaches from China were not included. Therefore, the diversity of the cultivars surveyed was limited. China is both origin centre of peaches and richness in germplasm of peaches, but only a few studies on diversity in peaches involved in less cultivars. Genetic diversity and genetic structure for a large amount from within and out of China and demes of peaches have not been studied.

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Although demes of peaches have no clear classifying criteria, this study divided edible peaches among crisp peach, honey peach, juicy peach, yellow peach, flat peach, nectarine according to Wang's research results (1990) with enzyme survey, and shouxingtao, bitao, weeping peach, red leaf peach for ornamental peaches classified by Zhang's observation (ISHS 404). The study aimed to analyze genetic diversity and genetic structure for the above mentioned 10 demes of 180 peaches using RAPD technique in order to conserve and utilize resources of peaches more effectively in accordance to molecular results.

2. Materials and methods

2.1. Materials

One hundred and eighty cultivars belonged to crisp peach, honey peach, juicy peach, yellow peach, flat peach, nectarine, shouxingtao, bitao, weeping peach, red leaf peach were used as the experimental materials (Table 1). Young leaf samples were collected from Zhengzhou Fruit Tree Institute, CAAS, Zhengzhou, China; Beijing Forestry and Fruit Tree Research Institute, Beijing, China; Jiangsu Provincial Horticultural Research Institute, Nanjing, China and some places in Wuhan, China. The leaves were washed with sterile distilled water, dried with clean absorbent paper, and then put into plastic bags and stored in −72 °C refrigerator for use.

2.2. DNA extraction

One or two young leaves with veins cut were put into sterile mortar, adding a little silica sand and 700 μl abstracting liquid (100 mmol l^{-1}. Tris–HCl (pH 8.0), 50 mmol l^{-1} EDTA (pH 8.0), 500 mmol l^{-1} NaCl, 10 mmol l^{-1}, β-mercaptoethanol, 3%SDS). After leaves were grounded with pestle, mixed liquid was put into 1.5 ml centrifuge tube, incubated in 65 °C water for 30 min, and then centrifuged for 2 min (speed 19,800 × g, below as the same). Upper liquid was transferred to another sterile centrifuge tubes, adding equal volume of phenol–chloroform (1:1) to the tube, gently inverted up and down for several times and centrifuged for 5 min. The other step was repeated as above. The upper aqueous phase was transferred to the other sterile centrifuge tubes, adding a drop of 5 mol l^{-1} NaAc and equal volume of isopropanol. The tubes were gently inverted up and down, then being static for a while and centrifuged for 2 min. Discarding liquid of the tubes, DNA precipitates were washed with 75% cold alcohol, then adding absolute ethanol. After being dried, the DNAs were dissolved with 100 μl TE and 5 μl RNAse (5 mg μl^{-1}), incubated for 30 min in 37 °C water. Centrifuged process was repeated once more. Finally, DNA was dissolved with 100 μl TE, and concentration measured by violet photometer. The original liquid was diluted to 10 ng μl^{-1} and stored at 4 °C in the refrigerator for further use.

2.3. PCR amplification

Twenty-two 10-base primers selected from 200 arbitrary primers were used for PCR amplification (Table 2). Qualified

Table 1
Materials used in this study

Demes	Scientific name	Common name
Shouxingtao	*Prunus persica* var. *densa*	Hongchongban
		Shoufen
		S1
		S2
		S9
		Shoubai
		Baidanban
		Fenshouxing
		Shouxintao(Pink)
		Shouxintao(Red)
Bitao	*P. persica* var. *duplex*	Jiangtao
		Shahongtao
		Wubaotao
		Renmiantao
		Feitao
		Honghuabitao
		Bitao
		Hongbitao
Weeping peach	*P. persica* var. *pendula*	Cuizhitao
		Yuanyangcuizhi
		Hongcuizhi
		Zhufencuizhi
		Cuizhitao
Red leaf peach	*P. persica* var. *vulgaris*	Hongyetao
		Luogehongye
		Hongye(jiangshu)
		Danbanzhitao
		Tskuba No. 3
		Tskuba No. 2
		Tskuba No. 6
		Zhiyetao
		Hongye(Huanong)
Crisp peach	*P. persica* var. *vulgaris*	Xuanchengtiantao
		Datiantao
		Shuibaitao
		Yangquanroutao
		Yibai
		Zhongzhoubaitao
		Luling
		Pingbaizi
		Tianjinshuimi
		Dunhuangdongtao
		Qinglingdongtao
		Wuyuexian
		Yunshu No.1
		Diaozhibai
		Yixianhong
		Lingbai No.7
		Dahongpao
Honey peach	*P. persica* var. *vulgaris*	Hanglumi
		Taiyuanshuimi
		Qiumi
		Qingzouhongpimitao
		Feichenghongli No.6
		Shenzoushuimi
		Qingzoubaipimitao
		Shenzouliheshuimi
		Wenzoushuimi
		Gegu
		Shenzouhongmi
		Feichenghonglitao

Table 1 (*Continued*)

Demes	Scientific name	Common name
		Changlixuetao
		Shenzoubaimi
Juicy peach	*P. persica* var. *vulgaris*	Yuhualu
		Spring time
		Zhongshanzhaolu
		Sunagowase
		Nunomiwase
		Zhaoxialu
		Shaji No.2
		Rebin
		Hakkobi
		1#2-1
		Ribenbaitao
		Hu No. 021
		Yinghualu
		V1-13
		Baihua
		Qungfeng
		Jingshanshuimi
		Xupudayulu
		Shnagshandayulu
		Fenghuayulu
		Runzoushuimi
		Zouyehuanglu
		Hu No.022
		Maixiang
		Mangxialu
		Zhaohui
		Tasubanawase
		1#2-6
		Chunlei
		Zhaoxia
		Zhaoxiangyi
		Guizoushuimi
		Jingyu
		Okayama No.3
		Shanghaishuimi
		Zhongbai No.8
		Yulu
		Wanshuomi
		Okayamahaku
		Akatsuki
		Zhaofeng
		Haban
		Mukai
		Zhaoyian
		Yingshuang
		Okubo
		Hakuho
		Xiahui No. 2
Flat peach	*P. persica* var. *platycarpa*	Chengpupantai
		Yuluoantao
		Jiaqing
		Xinhongzhaopantao
		Baimang
		Sulianpantao
		Lihepantao
		Yangzou No. 124
		Meiguopantao
		Fenghuapantao
		Chnagshengpantao
		Wuyuexianbiangan
		Zhaopantao

Table 1 (*Continued*)

Demes	Scientific name	Common name
Nectarine	*P. persica* var. *necturina*	NJN72
		Aimila
		Ruiguang No. 3
		Redsun
		Ruiguang No.
		Kala
		Huagang
		Shuguang
		Hongliguang
		Legrand
		Mayfire
		Earlyred No. 2
		Okitsu
		Baoyou No. 2
		Rayal Point
		Armking
		Fantasia
		Changliyoutao
		Shuho
Yellow peach	*P. persica* var. *vulgaris*	Red heaven
		Kan No. 5
		Elberta
		Tuscan
		Fertinimoroteini
		Phillips
		Dixired
		Favolate No. 3
		Dalihehuangrou
		Fayette
		Halford
		Chengxiang
		Lianhuang
		Jinxiu
		Jingcheng
		Fenghuang
		Huangnianhe
		Triumph
		Zhenhuang No. 4
		Flordking
		Zhenhuang No. 2
		Xizhuang No. 1
		Chengyan
		Mingxing
		Xiangjiaotao
		Zhanghuang No. 7
		Babygold No. 6
		Babygold No. 5
		Shilinghuangrou
		Luxiang
		Zhaohuangjing
		Linghuang No. 9
		NJC96
		Nong1-2-4
		Yunhuang
		Oroa
		Gold queen

was amplified condition, which contained PCR reaction total volume 25 μl composed of 10× PCR buffer 2.5 μl, $MgCl_2$ (25 mmol l^{-1}) 2.0 μl dNTP (2 mmol l^{-1}) 2.5 μl, Tag polymerase (5 U μl^{-1}) 0.24 μl, primer (16.5 ng μl^{-1}) 2.0 μl, template DNA (10 ng μl^{-1}) 5.0 μl. Primers were purchased from Shanghai Shengong Biological Engineering Company,

Table 2
Primers with arbitrary sequence in the RAPD analysis

Primer no.	Sequence of primers	Primer no.	Sequence of primers
S17	AGGGAACGAG	S125	CCGAATTCCC
S18	CCACAGCAGT	S167	CAGCGACAAG
S21	CAGGCCCTTC	S319	TGGCAAGGCA
S24	AATCGGGCTG	S341	CCCGGCATAA
S29	GGGTAACGCC	S360	AAGCGGCCTC
S43	GTCGCCGTCA	S441	GGCACGTAAG
S51	AGCGCCATTG	S444	AAGTCCGCTC
S60	ACCCGGTCAC	S452	CAGTGCTGTG
S65	GATGACCGCC	S459	GGTGCACGTT
S68	TGGACCGGTG	S464	GTGTCTCAGG
S118	GAATCGGCCA	S2134	AACACACGAG

and PCR buffer, Tag polymerase, $MgCl_2$ and dNTP all were from Shanghai Promega Company. After reaction liquid was mixed, a folium of mineral oil was used on the top. Amplification apparatus was DNA Thermal Cycler 480 (the Perkin-Elmer Corporation, USA). Reaction procedure was as follow: 93 °C 2 min, 36 °C 1 min, 72 °C 2 min, 1 cycle; 93 °C l min, 36 °C l min, 72 °C 2 min, 42 cycles; 93 °C 1 min, 36 °C 1 min, 72 °C 10 min, 1 cycle. After amplification, DNA fragments were analyzed by gel electrophoresis in 1.4% agarose (Spain) (to which 0.5 ug ml^{-1} ethidium bromide was added). Lambda DNA/*Eco*RI + *Hind*III (Huamei) and Gene Ruler™ 100 bp DNA Ladder Plus (MBI) were used as standard markers to estimate the approximate molecular weight of the amplified products.

2.4. Recorded data and statistic analyses

Resultant bands were screened and photographed under UV light. All reactions were repeated more than two times, and only bands which were bright and reproducible were recorded for analyses. Presence or absence of a band was coded by 1 or 0, respectively. Na (Observed number of alleles), Ne (Effective number of alleles), H (Nei's gene diversity), I (Shannon's information index) were calculated using POPGENE (Yeh and Yang, 1999); for genetic diversity and distance analyses, and dendrogram was constructed with based Nei's (1972) genetic distance. Genetic variance components within and among the demes were calculated with AMOVA (Excoffier, 1995).

3. Results and analyses

3.1. Diversity of different peach demes

DNAs of 180 accessions in 10 groups in *Prunus persica* were amplified with 22, 10-base primers (Table 2) selected from 200 arbitrary primers using RAPD technique. One hundred and eighty loci were observed and recorded. Polymorphism of demes was calculated with statistical analyses of the data by POPGENE (Table 3).

The numbers of alleles (Na) in all loci ranged from 1.20 to 1.73 with the mean of 1.42 among the 10 demes. The orders for different demes showed as yellow peach > honey peach > flat peach > juicy peach > red leaf peach > crisp peach > nectarine > bitao > shouxingtao > weeping peach. The effective alleles (Ne) in all loci changed from 1.1481 to 1.3953 with the mean of 1.26 for the demes, the orders of which showed as yellow peach > flat peach > honey peach > red leaf peach > crisp peach > bitao > shouxingtao > nectarine > juicy peach > weeping peach. Comparing average numbers of alleles with average effective numbers of alleles in all loci, there were big changes in orders of the demes except the highest and the lowest. The average Nei's gene diversity of the demese was 0.15, ranging from 0.082 to 0.237, and the orders as follow: yellow peach > honey peach > flat peach > red leaf peach > crisp peach > bitao > juicy peach > nectarine > shouxingtao > weeping peach. Shannon's information indexes of the demes were 0.22 ranging from 0.1184 to 0.3580. There was almost the same order for demes between Nei's gene diversity and Shannon's information indexes except juicy peach and bitao peach. From above mentioned four kinds of methods for analyzing genetic diversity, it can be concluded that Nei's gene diversity or Shannon's information indexes is suitable for evaluation of diversity, so diversity of the demes is yellow peach > honey peach > flat peach > red leaf peach > crisp peach > bitao and juicy peach > nectarine > shouxingtao > weeping peach.

Table 3
Genetic polymorphism of different groups in *Prunus persica*

Demes	Na[a]	Ne[b]	H[c]	I[d]
Shouxingtao	1.328	1.224	0.126	0.184
Bitao	1.344	1.243	0.134	0.196
Weeping peach	1.200	1.148	0.082	0.118
Red leaf peach	1.428	1.311	0.173	0.252
Crisp peach	1.394	1.243	0.141	0.209
Juicy peach	1.500	1.312	0.181	0.268
Juicy peach	1.450	1.216	0.133	0.204
Flat peach	1.478	1.315	0.179	0.264
Nectarine	1.356	1.223	0.128	0.189
Yellow peach	1.728	1.395	0.237	0.358
Mean[e]	1.42	1.26	0.15	0.22

[a] Observed number of alleles.
[b] Effective number of alleles.
[c] Nei's gene diversity.
[d] Shannon's information index.
[e] Average number.

3.2. Genetic structure among the demes

One hundred and eighty loci of 10 peach demes were analyzed using AMOVA software, showing that 11.91 and 88.09% genetic variation existed within and among demes, respectively. Genetic variation was about eight times higher among than within demes.

3.3. Cluster analyses of different demes

Peach demes were clustered (Fig. 1) with data of amplified loci, calculating genetic similarity and genetic distance (Table 4). From view of dendrogram, it could be divided into five sections if the genetic distance of 0.0351 used as joint line, of which the biggest section which had 99% bootstrap value

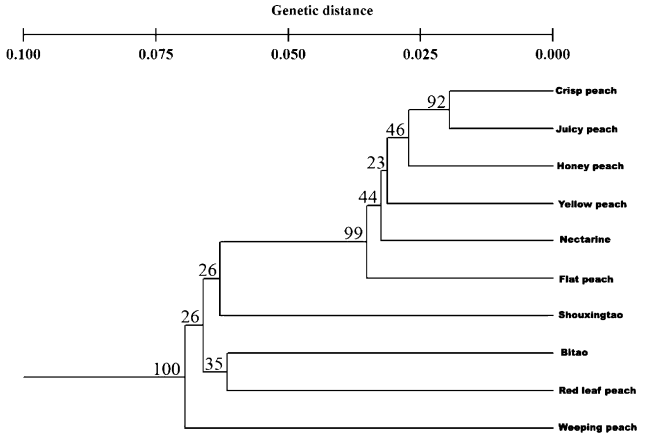

Fig. 1. Dendrogram Based Nei's (1972) Genetic distance. Bootstrap values with 1000 duplicates show above the branches.

included edible peaches, namely crisp peach, honey peach, juicy peach, yellow peach, flat peach, nectarine. The shortest distance in the section was 0.0195 between crisp peach and juicy peach; the other four sections contained shouxingtao, bitao, weeping peach, red leaf peach, which belonged to ornamental peaches, were distributed their own sections, respectively, and the longest genetic distance was 0.0694 between weeping peach and the other sections.

4. Discussion

The experiment was carried on crisp peach, honey peach, juicy peach, yellow peach, flat peach, nectarine and shouxingtao, bitao, weeping peach, red leaf peach about genetic diversity. Each deme has its own characteristics. Shouxingtao is dwarf, and has dense nodes on shoots, double buds on nodes generally; double are petals of bitao flowering before expanding of leaves; shoots of weeping peach are flexible and bending, having shot nodes and double buds on nodes; petals of red leaf peach with purple leaves are single; fruit of crisp peach is slightly round, crisp and tender; flesh of honey peach is hard melting, delicate, succulent, good character in store. Fruit of juicy peach with succulent juice is round or wide ovoid, top of which is round and obtuse and not suitable for store and traffic; Outline of flat peach is flat and unclear on seam; appearance of nectarine is hairless on fruit that is small in general. Fruit character of yellow peach is yellow or orange in both pulp and peel. Hairless on the surface of nectarine with yellow flesh and hairiness on the surface of yellow peach are basically distinguishable. Outlines of the demes become basis for conservation and use of peach germplasm. Modern molecular biological methods could provide more information for disclosing genetic diversity of peaches at DNA level. The combination combined morphology with molecular biology can give guidance for conservation and use for breeding. From analyses of diversity of different demes, Nei's gene diversity was generally consistent with Shannon's information index, both could be used for diverse analysis very well. Due to lack of available plant resources, the number of weeping peaches were smaller than other demes, therefore, the genetic diversity of weeping peach was slightly lower. Juicy peach had largest accessions in the experimented demes, while its diversity was ranked the 6th and less than red leaf peach with 9 accessions, flat peach with 13 accessions, juicy peach with 12 accessions, crisp peach with15 accessions and yellow peach with 36 accessions which had highest diversity. Diversity was not absolutely positive proportional to the experimented numbers of demes. From the results of the experiment, yellow peach had highest diversity which also conformed to the results, for example, yellow peach Nong 1-2-4 from south western China and yellow peach Xizhuang No. 1 from north western China were distinctly different from other peaches by checking of appearance of pollen and bands of enzymes (Wang's research, 1990; Wang and Zhou, 1990); Zong et al. (1995) revealed a result that Xizhuang had special SDS protein bands; Bruce et al. (1990) pointed out that yellow peach named Redhaven had the same band types with *P. persica* ssp. *ferganensis*. Hence, conservation numbers of yellow peach should be reserved more than any of other demes. Utilization of yellow peach for breeding is higher rate of variation than others. Other demes also could be reserved and used by the reasonable methods.

Genetic variation within and among the experimented demes was apparently different. It means that we should pay attention to conserving the amount of cultivars within demes so that diversity could be reserved mostly. We had done some analyses about core germplasm in each deme using the whole 180 accessions and listed important accessions within demes such

Table 4
Genetic identity and genetic distance among demes

Demes	SH	BI	WE	RE	CR	HO	JU	FL	NE	YE
SH	****	0.9398	0.9317	0.9298	0.9321	0.9252	0.9475	0.9380	0.9466	0.9450
BI	0.0621	****	0.9370	0.9403	0.9302	0.9235	0.9445	0.9285	0.9343	0.9374
WE	0.0707	0.0651	****	0.9160	0.9324	0.9266	0.9512	0.9307	0.9341	0.9370
RE	0.0728	0.0616	0.0877	****	0.9354	0.9358	0.9475	0.9361	0.9332	0.9501
CR	0.0704	0.0724	0.0700	0.0668	****	0.9739	0.9806	0.9633	0.9719	0.9660
HO	0.0777	0.0796	0.0762	0.0663	0.0264	****	0.9723	0.9653	0.9621	0.9701
JU	0.0539	0.0571	0.0500	0.0540	0.0195	0.0280	****	0.9728	0.9709	0.9714
FL	0.0640	0.0742	0.0718	0.0661	0.0374	0.0354	0.0276	****	0.9586	0.9676
NE	0.0548	0.0679	0.0682	0.0692	0.0285	0.0386	0.0296	0.0423	****	0.9675
YL	0.0566	0.0647	0.0651	0.0512	0.0346	0.0303	0.0290	0.0330	0.0330	****

SH, BI, WE, RE, CR, HO, JU, FL, NE, YE short for shouxingtao, bitao, weeping peach, red leaf peach, crisp peach, honey peach, juicy peach, flat peach, nectarine, yellow peach, respectively. *Notes*: Nei's genetic identity (above asterisks diagonal) and genetic distance (below asterisks diagonal).

as Hongchongban, Shouxintao (Pink) in the shouxingtao group; Hongbitao, Honghuabitao, Renmiantao in bitao group; Zhubo 3, Zhubo 6, Luogehongye in the red leaf peach group; Dahongpao, Wuyuexian, Qinglingdongtao, Yixianhong, Yangquanroutao, Datiantao in the crisp peach group; Qiumi, Taiyuanshuimi, Shenzhouhongmi, Wenzhoushuimi in the honey peach group; Okayamahaku, Spring time, Rebin, Hakuho, Xiahui No. 2, Zhaoyian, Fenghuayulu in the juicy peach group; Yulupantao, Wuyuexianbiangan, Sulianpantao, Jiaqing, Yangzhou 124 in the flat peach group; Shuguang, Ruiguang No. 2, Mayfire, Aimila, Hongliguang, Armking in the nectarine group; Xizhuang No. 1, Nong 1-2-4, Gold queen, Red haven, Jinxiu, Chengxiang, Elberta, Jingcheng in the yellow peach group(Cheng et al., 2002a,b,c; Cheng et al., 2003; Cheng, 2003a,b,c,d; Cheng, 2004). On the other hand, genetic recombination among demes, especially using higher diversity deme like yellow peach group, could produce more new types in order to satisfy selections for new cultivars.

The cluster result showed that there was a higher similarity among the the edible peach demes. The possible explanation might be that frequent gene flow from one deme to another occurred because of the crossing for new cultivar breeding in the history. On the other hand, the ornamental species were clustered into separate sections, because there were few chances of crossing between these demes and the gene exchange was limited. It is possible to gain new cultivars or to create novel germplasm of peach through hybridization between edible peaches and ornamental species. In fact, the cultivar Huayulu was bred by crossing and is a new genotype which could be used as edible peach as well as ornamental species (Wang et al. 2001).

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