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Genetic diversity and relationship among peach cultivars based on Random Amplified Microsatellite Polymorphism (RAMP)

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Abstract. Peach (*Prunus persica* (L.) Batsch) is a common fruit tree species, mainly in the temperate region. It is an important economic plant in Taiwan. In the present study, the genetic relationships among 26 cultivars of common peach (*P. persica* var. *vulgaris* Maxim.), 12 cultivars of nectarine (*P. persica* var. *nectarina* Maxim.), and three cultivars of flat peach (*P. persica* var. *platycarpa* Bailey) were estimated using RAMP markers. Eighty-two polymorphic bands were obtained using ten combinations of primers. The cluster analysis based on RAMP data revealed that the groupings were generally consistent with the classification of the varieties and the regions of origin of cultivars. The common peach cultivars originating in China and Japan formed a cluster. A possible explanation is that the Japanese cultivars may be developed from cultivars introduced from China. Within flat peach cultivars, the groupings also indicated that the genetic relationship among cultivars is correlated with the regions of origin of cultivars. Analysis of molecular variance (AMOVA) revealed that the variance components among and within three peach groups expressed as percentages of the total variation were 30.3% and 69.7%, respectively.

Keywords: Peach; *Prunus persica*; Genetic relationship; RAMP; AMOVA.

Introduction

Peach (*Prunus persica* (L.) Batsch) is a common fruit tree in temperate regions. It is considered the queen of temperate-zone fruits and, next to apple, is the world's most widely grown fruit tree (Bailey and Bailey, 1976). Three varieties can be recognized taxonomically based on fruit morphology. The common peach (*P. persica* var. *vulgaris* Maxim.) has rounded and hairy fruits. The nectarine (*P. persica* var. *nectarina* Maxim.) has rounded fruits without hairs. The flat peach (*P. persica* var. *platycarpa* Bailey) has flat fruits. Peach is one of the important economic plants in Taiwan. Besides traditional cultivars, many cultivars of common peach and nectarine were introduced from Japan and United States recently and grown in high altitude areas. The over-utilization of high altitude mountain slopes resulted in soil erosion and the destruction of natural environments. Peach breeding programs in Taiwan, aimed at producing cultivars suitable for lower altitudes, could reduce the destruction of natural environments. Molecular markers such as isozymes (Arulsekar et al., 1986; Durham et al., 1987; Chaparro et al., 1994), RFLP (Eldredge et al., 1992; Rajapakse et al., 1995; Quarta et al., 1996), and RAPD (Chaparro et al., 1994; Rajapakse et al., 1995; Warburton et al., 1996), have been useful in estimating the genetic relationship and in the genetic linkage mapping of the peach genome. The estimation of genetic relationships among cultivars provides the basic information for breeding programs. Molecular markers could also be used in assisting the process of artificial selection. Random amplified microsatellite polymorphism (RAMP; Wu et al., 1994) has been demonstrated to be another potentially valuable molecular marker for the study of genetic relationships in cultivated plant species. The combination of a simple sequence repeat (SSR; microsatellite) and a random sequence was used to amplify genomic DNA fragments in RAMP. RAMP has been employed in studies of the cultivars of barley (Wu et al., 1994; Becker and Heun, 1995; Sanchez de la Hoz et al., 1996). The usefulness of the RAMP molecular marker has not been widely tested in other plant species. The objectives of the present study are to investigate the genetic relationship of some peach cultivars available in Taiwan and to estimate the genetic diversity among and within major peach groups using RAMP markers.

Materials and Methods

The leaf samples of 41 peach cultivars (Table 1) were collected from the Lona division of the Taiwan Agriculture Research Institute and Mountain Experimental Farm of National Taiwan University and stored at -70°C before DNA extraction. DNA extraction followed the method of Doyle and Doyle (1990). The DNA concentration was measured using a Hoefer TKO 100 fluorometer with Hoechst dye solution. Ten combinations of primers selected between three SSR-primers (RM21, RM23, RM24) and five random primers (A1, A4, B1, Q5, V6; Operon Tech. Inc., USA) were used for RAMP amplifications. The five random primers were also used singularly for RAPD amplifi-

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cations under the same conditions as RAMP. RAMP markers are the bands resulting from the subtraction of the bands of RAPD amplification from the bands of RAMP amplification (Wu et al., 1994). The nucleotide sequences of these SSR and random primers together with the primer combinations used in the present study are listed in Table 2. Reactions were performed in 25 μL volumes containing 1x reaction buffer (12.5 mM Tris-HCl, 1.9 mM $MgCl_2$, 62.4 mM KCl, gelatin 0.12% (w/v), Triton X-100 1.2% (w/v)), 120 μM dATP, dGTP, dTTP, dCTP, 0.2 μM primer, 0.2 units Taq DNA polymerase (HT Biotechnology) and 5 ng of genomic DNA. A thermocycler (Model PTC-100, MJ Research, USA) was programmed for an initial denaturation of 1 min at 94°C followed by one cycle of 150 s at 92°C, 1 min at 40°C, and 2 min at 72°C and 45 cycles of 1 min at 92°C, 1 min at 40°C, and 2 min at 72°C. The amplification was completed after 5 min at 72°C. Reaction products were separated by electrophoresis (Model P9DS, Owl Scientific, Inc., USA) in 7% polyacrylamide gel at 300 V for 130 min at 1°C in 1x TBE buffer and visualized by silver staining (Plusone TM DNA Silver Staining Kit; Pharmacia Biotech Inc., Sweden). A molecular size marker (pGEM DNA Markers, Promega Corporation) was used on each run.

Table 1. Peach cultivars studied.

Common peach	Nectarine
Originated in Japan	Originated in Japan
1 Nakatsu Hakuto	27 Wase nectarine
2 Sunago wase	28 Wase nectarine 10-16
3 Okubo	29 Wase nectarine 19-21
4 Nishino Hakuto	30 Okitsu
5 Kansuke Hakuto	
6 Aichi Hakuto	Originated in USA
7 Asama Hakuto	31 New Yorker
8 Kawanakashima	32 Flavortop
9 Benishimizu	33 Fantasia
10 Sedouchi	34 Nectared 4
11 Odama Hakuto	35 Nectared 5
12 Hachiban Hakuto	36 Nectared 6
13 Shiga Hakuto	37 Nectared 8
14 Nagano wase	38 Nectared 9
15 Kodaira wase	
16 Suzuki Hakuto	
17 Matsumori wase	
18 Abe Hakuto	
19 Nagazawa Hakuto	
	Flat peach
Originated in China	Originated in China
20 Qiu Bai Tao	39 San Hua Huon Banto
21 Shen Zhou Mi Tao	
22 Shen Zhou Bai Xue	Originated in Japan
23 Shang Hai Shui Mi Tao	40 Yaezaki Banto
24 Fei Chang Tao	41 Akabana Banto
Originated in other countries	
25 Redhaven (USA)	
26 Tai Non Ten Mi Tao, a sport of Premier (Brazil)	

The presence or absence of 82 polymorphic and reproducible RAMP markers were scored for each cultivar. The data were used to calculate a similarity matrix among cultivars employing Dice (1945) algorithm. The similarity matrix was employed in the UPGMA cluster analysis. In the analysis of molecular variance (AMOVA; Excoffier et al., 1992), the Dice similarity was converted into distance by subtracting similarity from one, and then the variance components among and within common peach, nectarine, and flat peach groups were calculated from the distance matrix. The variance components were expressed as percentages, and the significance of each variance component was tested by 10,000 random permutations. The Φ_{st} distance matrix among peach groups derived from AMOVA was used in a UPGMA cluster analysis and a principal coordinate analysis.

Results and Discussion

All Cultivars Included

An example of the polymorphism detected among some test samples using SSR primer RM23 and random primer OPV6 is shown in Figure 1. The similarity matrix among peach cultivars based on 82 polymorphic RAMP markers is shown in Table 3. The dendrogram (Figure 2) derived from this similarity matrix revealed that the groupings of common peach were generally consistent with the classification of varieties and the regions of the origin of cultivars. The common peach cultivars originating in China (codes 20-24) and Japan (codes 1-19) formed a cluster while the remaining cultivars (codes 25 and 26) distantly linked to the cluster of nectarine (codes 27-38) and flat peach (codes 39-41). All three cultivars of the flat peach were linked together as a small group before being joined by the nectarine cultivars “Okitsu” (code 30) and “New Yorker” (code 31). Other cultivars of nectarine and a common peach cultivar “Tai Non Ten Mi Tao” (code 26) were linked together as a larger group. Common peach originating in China and Japan did not separate into two distinct clusters. The cophenetic correlation coefficient of this cluster analysis was 0.80.

Common Peach Cultivars Only

When 26 cultivars of common peaches only were considered, the dendrogram (Figure 2) indicated that cultivars originating in China and Japan were linked as a cluster without separating into two groups. A possible explanation is that the Japanese cultivars may be developed from cultivars introduced from China. Cultivars “Redhaven” (codes 25) and “Tai Non Ten Mi Tao” (code 26) that showed relationship with nectarine cultivars were the two most isolated cultivars. Cultivar “Redhaven” originated in the United States while cultivar “Tai Non Ten Mi Tao” is a sport of cultivar “Premier”, which was introduced from Brazil. Cultivars “Okubo” (code 3), “Nishino Hakuto” (code 4), and “Odama Hakuto” (code 11) of Japan have the closest relationship. Cultivar “Nishino” was derived from a seedling in a mixed planting of “Okubo” and

"Hakuto" (Yoshida, 1991). This close genealogical relationship was observed in the dendrogram. Other cultivars showing close relationships include "Abe Hakuto" (code 18) and "Nagazawa Hakuto" (code 19); "Nagano Wase" (code 14) and Kodaira Wase" (code 15); "Aichi Hakuto" (code 6) and Asama Hakuto" (code 7). Although we do not know the genealogy of these cultivars, the dendrogram showed a close relationship between two pairs of "Hakuto" (which means white peach in Japanese) and one pair of "Wase" (which means early fruiting in Japanese).

Nectarine Cultivars Only

When 12 cultivars of nectarine only were considered, the dendrogram (Figure 2) indicated that cultivars "Nectared 4" (code 34), "Nectared 5" (code 35), "Nectared 6" (code 36),"Nectared 8" (code 37), and "Nectared 9"

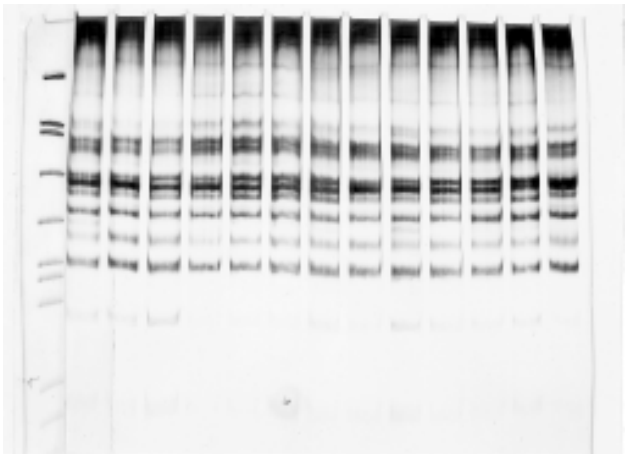

Figure 1. An example of the polymorphism detected among some test samples using ISS primer RM23 and random primer OPV6. (left to right: lane 1, 100 bp marker; lane 2 - lane 14, cultivar codes 27, 34-38, 28-33, and 40).

Table 2. Nucleotide sequences of the primers and primer combination used.

Primer	Sequence
SSR primer	
RM21	5'-CTCCGCCGCCG-3'
RM23	5'-GGCACCACCAC-3'
RM24	5'-GCAACAACAAC-3'
Random primer	
OPA1	5'-CAGGCCCTTC-3'
OPA4	5'-AATCGGGCTG-3'
OPB1	5'-GTTTCGCTCC-3'
OPQ5	5'-CCGCGTCTTG-3'
OPV6	5'-ACGCCCAGGT-3'
Primer combination	
A1/RM23	Q5/RM23
A4/RM23	Q5/RM24
A4/RM24	V6/RM21
B1/RM23	V6/RM23
B1/RM24	V6/RM24

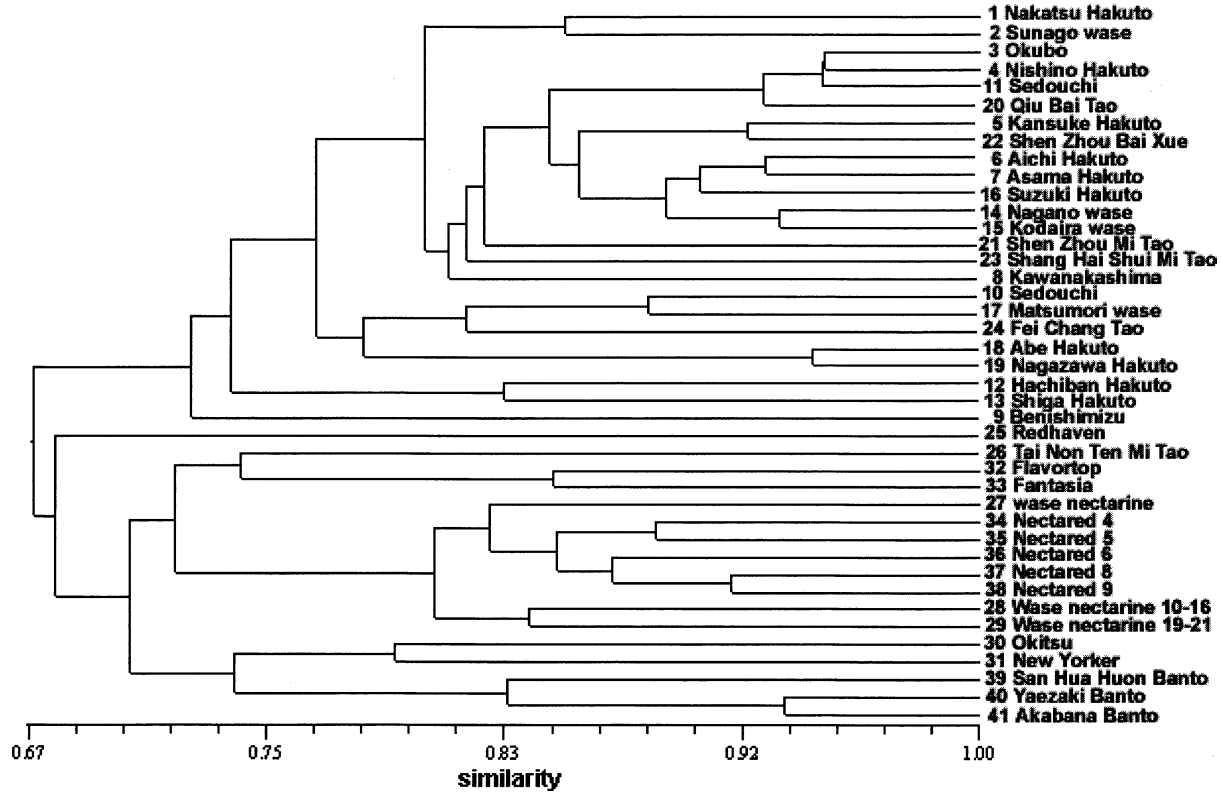

Figure 2. Dendrogram of all peach cultivars studied based on polymorphic RAMP markers.

Table 3. Simility matrix among peach cultivars based on polymorphic RAMP bands.

cultivar codes	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	29	30	31	32	33	34	35	36	37	38	39	40	41
1	1.000																																								
2	0.854	1.000																																							
3	0.846	0.847	1.000																																						
4	0.824	0.826	0.945	1.000																																					
5	0.750	0.777	0.846	0.843	1.000																																				
6	0.843	0.844	0.873	0.889	0.863	1.000																																			
7	0.800	0.804	0.870	0.830	0.840	0.925	1.000																																		
8	0.763	0.788	0.800	0.816	0.804	0.854	0.851	1.000																																	
9	0.659	0.652	0.711	0.727	0.756	0.750	0.721	0.723	1.000																																
10	0.698	0.688	0.766	0.783	0.884	0.804	0.778	0.782	0.784	1.000																															
11	0.863	0.844	0.945	0.944	0.863	0.870	0.849	0.816	0.750	0.804	1.000																														
12	0.747	0.769	0.696	0.719	0.795	0.764	0.742	0.690	0.686	0.730	0.719	1.000																													
13	0.737	0.784	0.777	0.772	0.842	0.752	0.747	0.708	0.617	0.729	0.772	0.833	1.000																												
14	0.796	0.781	0.830	0.846	0.837	0.904	0.863	0.848	0.714	0.805	0.827	0.706	0.722	1.000																											
15	0.825	0.827	0.857	0.854	0.866	0.913	0.871	0.837	0.747	0.828	0.854	0.786	0.771	0.929	1.000																										
16	0.825	0.827	0.857	0.835	0.887	0.913	0.891	0.857	0.771	0.841	0.874	0.786	0.750	0.869	0.918	1.000																									
17	0.708	0.757	0.750	0.745	0.833	0.804	0.800	0.845	0.732	0.884	0.765	0.723	0.716	0.816	0.845	0.804	1.000																								
18	0.742	0.729	0.722	0.716	0.719	0.779	0.817	0.778	0.720	0.810	0.758	0.734	0.659	0.791	0.822	0.800	0.809	1.000																							
19	0.739	0.727	0.740	0.735	0.739	0.776	0.813	0.796	0.744	0.805	0.755	0.732	0.681	0.787	0.839	0.796	0.848	0.941	1.000																						
20	0.835	0.855	0.919	0.936	0.835	0.862	0.822	0.827	0.742	0.753	0.917	0.725	0.745	0.819	0.827	0.846	0.757	0.729	0.747	1.000																					
21	0.723	0.752	0.824	0.840	0.830	0.840	0.776	0.758	0.750	0.782	0.820	0.691	0.731	0.833	0.863	0.800	0.787	0.667	0.711	0.832	1.000																				
22	0.796	0.800	0.868	0.846	0.918	0.904	0.843	0.747	0.762	0.818	0.846	0.776	0.804	0.840	0.869	0.848	0.816	0.703	0.723	0.838	0.833	1.000																			
23	0.755	0.743	0.830	0.788	0.796	0.865	0.882	0.768	0.643	0.809	0.827	0.698	0.680	0.820	0.828	0.869	0.755	0.769	0.745	0.781	0.750	0.800	1.000																		
24	0.652	0.687	0.720	0.735	0.783	0.796	0.729	0.731	0.795	0.857	0.714	0.684	0.681	0.809	0.817	0.774	0.783	0.729	0.705	0.687	0.800	0.809	0.702	1.000																	
25	0.584	0.646	0.660	0.653	0.674	0.674	0.667	0.644	0.640	0.625	0.695	0.597	0.591	0.659	0.711	0.711	0.652	0.634	0.659	0.646	0.667	0.659	0.637	0.682	1.000																
26	0.591	0.632	0.604	0.617	0.614	0.617	0.565	0.584	0.514	0.691	0.617	0.560	0.598	0.644	0.629	0.629	0.682	0.593	0.595	0.589	0.651	0.622	0.578	0.643	0.568	1.000															
27	0.680	0.731	0.743	0.718	0.680	0.777	0.792	0.735	0.602	0.636	0.738	0.612	0.604	0.768	0.755	0.755	0.701	0.711	0.667	0.712	0.632	0.687	0.727	0.645	0.711	0.629	1.000														
28	0.696	0.747	0.740	0.755	0.630	0.776	0.771	0.753	0.634	0.627	0.755	0.642	0.659	0.766	0.753	0.710	0.717	0.729	0.727	0.727	0.689	0.702	0.681	0.682	0.706	0.667	0.817	1.000													
29	0.714	0.800	0.736	0.750	0.673	0.769	0.725	0.727	0.612	0.607	0.750	0.667	0.660	0.760	0.788	0.747	0.714	0.681	0.723	0.762	0.750	0.700	0.660	0.681	0.725	0.622	0.747	0.842	1.000												
30	0.645	0.680	0.693	0.687	0.645	0.687	0.680	0.596	0.675	0.635	0.707	0.561	0.565	0.653	0.660	0.681	0.645	0.651	0.674	0.680	0.615	0.653	0.632	0.629	0.674	0.659	0.745	0.711	0.695	1.000											
31	0.558	0.602	0.596	0.630	0.558	0.630	0.600	0.644	0.740	0.623	0.630	0.533	0.518	0.591	0.575	0.575	0.628	0.633	0.634	0.624	0.571	0.568	0.523	0.659	0.633	0.615	0.621	0.723	0.636	0.795	1.000										
32	0.596	0.653	0.686	0.700	0.532	0.660	0.633	0.611	0.617	0.612	0.680	0.530	0.538	0.667	0.653	0.611	0.638	0.690	0.667	0.693	0.652	0.604	0.625	0.667	0.621	0.698	0.716	0.791	0.708	0.681	0.714	1.000									
33	0.607	0.674	0.624	0.681	0.581	0.681	0.659	0.644	0.632	0.684	0.652	0.564	0.565	0.719	0.652	0.607	0.682	0.699	0.667	0.667	0.667	0.629	0.629	0.729	0.543	0.785	0.667	0.753	0.711	0.744	0.759	0.851	1.000								
34	0.625	0.699	0.712	0.706	0.667	0.725	0.700	0.701	0.610	0.667	0.706	0.612	0.653	0.714	0.722	0.742	0.667	0.652	0.652	0.680	0.702	0.633	0.673	0.696	0.764	0.705	0.845	0.804	0.796	0.753	0.651	0.723	0.705	1.000							
35	0.584	0.674	0.645	0.659	0.628	0.723	0.681	0.621	0.640	0.634	0.652	0.564	0.588	0.674	0.652	0.652	0.636	0.627	0.619	0.624	0.690	0.652	0.629	0.706	0.667	0.709	0.778	0.786	0.756	0.814	0.709	0.690	0.767	0.886	1.000						
36	0.645	0.740	0.693	0.707	0.667	0.768	0.742	0.723	0.582	0.674	0.687	0.659	0.652	0.800	0.787	0.723	0.731	0.698	0.674	0.680	0.747	0.695	0.737	0.742	0.674	0.682	0.809	0.809	0.800	0.667	0.602	0.725	0.800	0.839	0.800	1.000					
37	0.673	0.743	0.755	0.750	0.694	0.788	0.765	0.727	0.643	0.667	0.731	0.667	0.701	0.780	0.788	0.747	0.735	0.703	0.702	0.705	0.771	0.740	0.720	0.766	0.747	0.711	0.828	0.851	0.840	0.737	0.659	0.771	0.787	0.898	0.854	0.905	1.000				
38	0.713	0.778	0.771	0.766	0.713	0.822	0.781	0.745	0.690	0.674	0.785	0.674	0.680	0.777	0.784	0.765	0.752	0.723	0.701	0.741	0.727	0.757	0.718	0.722	0.745	0.688	0.882	0.887	0.816	0.755	0.703	0.768	0.745	0.871	0.851	0.837	0.913	1.000			
39	0.624	0.680	0.713	0.707	0.645	0.707	0.701	0.660	0.675	0.667	0.707	0.585	0.630	0.674	0.723	0.681	0.731	0.721	0.719	0.680	0.659	0.695	0.632	0.674	0.698	0.706	0.723	0.800	0.737	0.733	0.747	0.813	0.744	0.753	0.744	0.733	0.779	0.796	1.000		
40	0.571	0.593	0.609	0.622	0.595	0.622	0.614	0.659	0.648	0.658	0.644	0.575	0.627	0.605	0.659	0.635	0.690	0.727	0.725	0.615	0.585	0.581	0.558	0.600	0.649	0.658	0.659	0.716	0.651	0.691	0.757	0.707	0.649	0.690	0.623	0.667	0.698	0.697	0.815	1.000	
41	0.541	0.587	0.624	0.615	0.612	0.615	0.652	0.651	0.667	0.701	0.659	0.514	0.571	0.621	0.674	0.651	0.706	0.744	0.741	0.609	0.578	0.598	0.598	0.617	0.692	0.649	0.698	0.732	0.644	0.732	0.773	0.747	0.667	0.706	0.641	0.659	0.690	0.711	0.854	0.932	1.000

Table 4. Variance components of common peach, nectarine, and flat peach groups based on RAMP data.

Source of variation	d.f.	SSD	MSD	Variance component	% Total variance	P-value*
Among groups	2	116.48	58.24	4.59	30.3	< 0.001
Within groups	38	401.20	10.56	10.56	69.7	< 0.001

*After 10,000 permutations.

(code 38) that have one or two common parents (Yamaguchi, 1985; Yoshida, 1991; Baird et al., 1992) were linked as a cluster. The Japanese "Wase nectarine" (code 27) showed a relationship with this cluster. Other cultivars showing close relationships include "Flavortop" (code 32) and "Fantasia" (code 33); "Wase nectarine 10-16" (code 28) and "Wase nectarine 19-21" (code 29). The cultivar "Okitsu" (code 30), which originated in Japan from "Precoce de Croncels" x "Lord Napier" (Okie, 1998), was most closely related to the cultivar "New Yorker" (code 31). The cultivars "Precoce de Croncels" and "Lord Napier" were not included in the present study.

Flat Peach Cultivars Only

Three cultivars of flat peaches were analyzed in the present study. Dendrogram (Figure 2) indicated that the Japanese "Yaezaki Banto" (code 40) linked to Japanese "Akabana Banto" (code 41) first and was then joined by the Chinese "San Hua Huon Banto" (code 39). The genealogy of these cultivars is unknown. However, the result also indicated that the genetic relationship among cultivars is correlated with the regions of cultivar origin.

AMOVA

The result of AMOVA (Table 4) indicated that the variance components expressed as percentages of the total variation among and within groups were 30.3% and 69.7%, respectively. The random permutation test revealed that both variance components were highly significant ($p < 0.001$). Considerable genetic differentiation was observed among three peach groups based on RAMP data, and a large percentage (30.3%) of the total variation was attributable to the difference among groups.

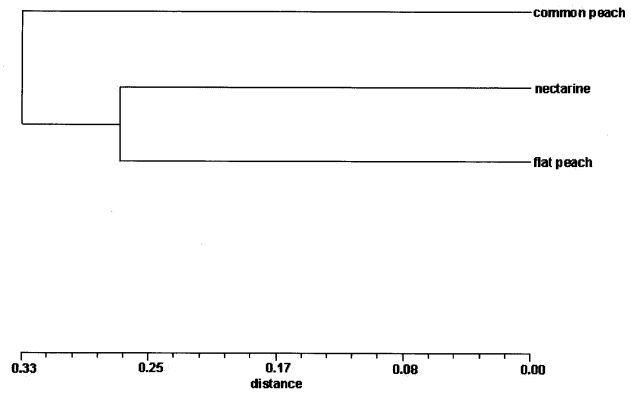

Figure 3. Dendrogram of peach varieties based on Φ_{st} distances.

Table 5. Φ_{st} distance matrix among common peach, nectarine, and flat peach groups.

	Common peach	Nectarine	Flat peach
Common peach	0.000		
Nectarine	0.282	0.000	
Flat peach	0.378	0.266	0.000

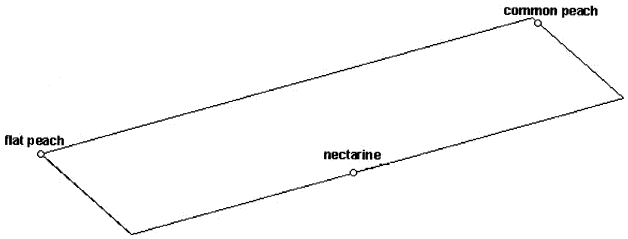

Figure 4. The result of principal coordinate analysis based on Φ_{st} distance matrix among groups.

Dendrogram Among Peach Groups

The Φ_{st} distances between groups (Table 5) were derived from AMOVA. The result of the cluster analysis based on Φ_{st} distance matrix is shown in Figure 3. The dendrogram indicated that nectarine and flat peach are closely related while common peach is a more isolated group. However, the cophenetic correlation coefficient of this cluster analysis was only 0.61, indicating a large distortion in the clustering. The result of principal coordinate analysis is shown in Figure 4. The three coordinates accounted for 100% of the total variation without any distortion. Nectarine is located between common peach and flat peach in this diagram. Table 5 shows that nectarine is also closely related to common peach with Φ_{st} of 0.282. The largest distance (0.378) exists between common peach and flat peach.

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應用逢機擴增微衛星多型性研究桃栽培品種間之遺傳歧異度及遺傳關係

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桃（*Prunus persica* (L.) Batsch）為一種常見溫帶果樹，在台灣是一種重要經濟作物。本研究應用逢機擴增微衛星多型性分子標記探討 26 個生食桃栽培品種、12 個油桃栽培品種及 3 個蟠桃栽培品種間之遺傳關係。採用 10 組引子組合得到 82 條多型性條帶，根據此等多型性條帶數據進行歸群分析，歸群結果顯示大致與變種分類及產地吻合。產自中國和日本的生食桃品種歸為一群，可能的原因是日本的品種乃由中國品種育種而成。三種蟠桃品種間的關係也顯示與產地有關。分子變方分析之結果顯示三群桃之群間和群內變方成分各佔總變方成分之 30.3% 和 69.7%。

關鍵詞：桃；薔薇科；遺傳關係；RAMP；AMOVA。