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Classification of Apricot Varieties by RAPD Analysis

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Summary

We carried out RAPD analysis for classifying apricot cultivars and related species, such as *P. sibirica* L. and *P. brigantina* Vill.

For this purpose, 225 Operon primers were screened by using five representative varieties and 18 primers which provide plural polymorphisms.

Using these primers, 33 varieties of *P. armeniaca* L. and two related species were tested for RAPD, then classified by cluster analysis and quantification method of the third type, based on the absence or presence of corresponding bands. The apricots were classified into two large groups by both analyses; "Western group" (A) and "Eastern group" (B). However, *P. sibirica* and *P. brigantina,* which are related to *P. armeniaca,* and two Chinese varieties, 'Bai-xing' and 'Ren-xing', did not belong to these two groups. Since RAPDs among Chinese apricots were very diverse, they were placed into both groups A and B. All Japanese apricots were classified into group B. Considering that Chinese apricots have large variations, we hypothesize that Chinese apricots may be the ancestors of Eastern and Western apricots.

Key Words: apricot, *Prunus,* RAPD, classification.

Introduction

The apricot belongs to the genus *Prunus,* and has several species. Cultivated apricots are mostly derived from *P. armeniaca* L. The distribution areas of *P. armeniaca* are wide and many ecotypes exist. Bailey and Hough (1975) classified cultivated apricots of North Africa, Europe, and Asia into the following six groups ; European, Irano-Caucasian, Central Asian, Dzhungar-Zailij, East Chinese, and North Chinese. In their classification, some apricots of the North Chinese group were included within *P. mandshurica* Koehne and *P. sibirica* L., whereas the East Chinese group included *P. ansu* Komar. They classified *P. ansu* as a distinct species, although some authors presently include it in *P. armeniaca.* The classification of apricots was previously based on morphological markers (Yoshida and Yamanishi, 1988), but recently isozyme markers have been used for this purpose (Byrne and Littleton, 1989). But, these methods do not provide enough polymorphisms to discriminate varieties and reveal a clear relationship among them.

Recently, molecular markers for classification have been developed by using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD). RFLP markers have been applied to construct genetic maps of peach (Eldrege et al., 1992), to study genetic variations of *Malus, Prunus,* and *Rubus* species (Nybom et al., 1990), and to identify cultivars of raspberry (Parent and Page, 1992) and Japanese pear (Teramoto et al., 1994). RAPD (Williams et al., 1990) or AP-PCR (Welsh et al., 1990) analysis has been established for molecular markers. This method can detect DNA polymorphisms more simply and rapidly even among closely related accessions. We have confirmed that RAPD analysis is useful for classification and cultivar discrimination in mume (Shimada et al., 1994). In this paper, we investigated the relationships among Japanese and European apricots by RAPD analysis.

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Materials and Methods

Plant materials

Thirty-three cultivated varieties of *P. armeniaca* and 2 related species were used in this study (Table 1). All materials were provided by Chiyoda experimental farm, Fruit Tree Research Station.

DNA isolation

Total DNA was isolated from fresh leaves of these materials by the method of Doyle and Doyle (1987) with a slight modification, that is, after the DNA is precipitated in isopropanol, the DNA pellet was redissolved in sterile water and reprecipitated in cold ethanol. DNA concentration was determined by the mini-

gel method (Sambrook et al., 1989) i. e. by comparing with standard lambda DNA, and that diluted to approximately 3 ng·μl^{-1} with sterile water.

PCR condition and DNA electrophoresis

PCR reaction was carried out in 10 μl mixtures containing 10 ng apricot genomic DNA, 2 μM primer, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 0.1 mM each of dATP, dTTP, dGTP, dCTP (Takara Biomedicals,Tokyo, Japan), and 0.2 unit of *Taq* DNA polymerase (*AmpliTaq*®, Perkin Ermer Cetus,Norwalk, CT, U.S.A.). Amplification was performed in a BioOven (BioTherm Co., Fairfax, VA, U.S.A.) programed for 45 cycles of 94 °C for 10 sec, 33 °C for 1 min., 73 °C for 2 min. The amplified products were separated by electrophoresis in 2% SeaKem® LE agarose (FMC, Rockland, ME, U.S.A.) gel with TAE buffer (0.04 M Tris-acetate containing 1 mM EDTA). The gel was then stained with ethidium bromide according to Sambrook et al. (1989), and photographed by Polaroid 665 under UV light.

Screening of primers

To detect DNA polymorphisms efficiently, screening was performed for 225 primers (Operon Co., Ltd., Alameda, CA, U.S.A. kit A-K). 'Niigataoumi', 'Kyoudaimaru', 'Bai-xing', 'Early Orange' and 'Nepal apricot', were selected as representatives because of their morphological diversities.

Data analysis

Polymorphic DNA fragments were scored on the basis of presence or absence of comparable bands among varieties. The number of non-shared bands between each pair of accessions was recorded for those fragments and subjected to the cluster analysis using group average method and quantification method of the third type (Hayashi, 1950) to construct the dendrogram and scatter diagram.

Results and Discussion

Screening of the primers

To detect RAPDs efficiently, we screened 225 Operon primers with different GC contents (12 primers for 40%, 14 primers for 50%, 143 primers for 60%, and 56 primers for 70%). In total, 1,542 DNA fragments were amplified and the fragment numbers per GC content were as follows; 31 fragments with 12 primers of 40% GC, 76 fragments with 14 primers of 50% GC, 1,015 fragments with 143 primers of 60% GC, and 420 fragments with 56 primers of 70% GC. Average amplified fragments and polymorphic DNA fragments per GC contents are shown in Table 2. As the concentration of G + C became higher, the number of amplified fragments per primer increased. The sequences of primers were not related to the numbers of the polymorphic DNA (data not shown), but we confirmed that higher GC contents produced high frequencies of RAPDs because of increases in total fragments. G + C content is an important index for the selection of primers because the G + C content of primer is also associated with the melting temperature (Tm) and related to the reproducibility of data (Fritsch et al.,1993). Out of these 225 primers, we selected 18 most appropriate primers based

Table 1. Plant materials.

Code	Name of materials	Scientific Name	Origin
1	Akita oumi	*P. armeniaca*	Japan
2	Aomorisan anzu	*P. armeniaca*	Japan
3	Heiwa	*P. armeniaca*	Japan
4	Hiroshima koanzu	*P. armeniaca*	Japan
5	Jinshirou	*P. armeniaca*	Japan
6	Koushiu oumi	*P. armeniaca*	Japan
7	Kyoudai maru	*P. armeniaca*	Japan
8	Mame-anzu	*P. armeniaca*	Japan
9	Mikanmomo	*P. armeniaca*	Japan
10	Mochi anzu	*P. armeniaca*	Japan
11	Niigata oumi	*P. armeniaca*	Japan
12	Nodokukuri	*P. armeniaca*	Japan
13	Ogasawara	*P. armeniaca*	Japan
14	Shimizugou	*P. armeniaca*	Japan
15	Shinshiu oumi	*P. armeniaca*	Japan
16	Takanomanjiu	*P. armeniaca*	Japan
17	Wase oumi	*P. armeniaca*	Japan
18	Yamagata 3	*P. armeniaca*	Japan
19	Bai-xing	*P. armeniaca*	China
20	Li-zi-xing	*P. armeniaca*	China
21	Mai-huang-zhun-xing	*P. armeniaca*	China
22	Mei-tao-xing	*P. armeniaca*	China
23	Ren-xing	*P. armeniaca*	China
24	Nepal apricot No. 85247	*P. armeniaca*	Nepal
25	Nepal apricot No. 85260	*P. armeniaca*	Nepal
26	Alexander	*P. armeniaca*	Russia
27	Hajihaliloulu	*P. armeniaca*	Turkey
28	Hasanbay	*P. armeniaca*	Turkey
29	Blenheim	*P. armeniaca*	Europe
30	Early Orange	*P. armeniaca*	Europe
31	Tilton	*P. armeniaca*	Europe
32	Goldcot	*P. armeniaca*	USA
33	Harcot	*P. armeniaca*	Canada
34	Alpine plum	*P. brigantina*	Alpus
35	Siberian apricot	*P. sibirica*	China

Table 2. The relationship between GC content and frequency of RAPDs.

GC content of primer	Total numbers of evaluated primers	Ave. of fragments/pri.	Ave. of RAPD/pri.
40 %	12	2.6	0
50 %	14	5.4	0.36
60 %	143	7.1	0.49
70 %	56	7.5	0.57

Table 3. G + C contents of selected primers.

code	Sequence	G + C content
1	5'-GACGGATCAG-3'	60 %
2	5'-TTCCCCCCAG-3'	70 %
3	5'-TGAGTGGGTG-3'	60 %
4	5'-GTTGCCAGCC-3'	70 %
5	5'-ACCGCGAAGG-3'	70 %
6	5'-CACCGTATCC-3'	60 %
7	5'-GGGGTGACGA-3'	70 %
8	5'-CCCAAGGTCC-3'	70 %
9	5'-TCACCACGGT-3'	60 %
10	5'-CACCAGGTGA-3'	60 %
11	5'-TTATCGCCCC-3'	60 %
12	5'-TGCCGAGCTG-3'	70 %
13	5'-AGTCGTCCCC-3'	70 %
14	5'-AGAGGGCACA-3'	60 %
15	5'-CCGCATCTAC-3'	60 %
16	5'-TGGTCACCGA-3'	60 %
17	5'-GTGCCTAACC-3'	60 %
18	5'-AGCGTGTCTG-3'	60 %

on the plural and reproducible RAPDs (Table 3). The G+C contents of these primers are 60% or 70%. Using these primers, we could always detect more than one RAPD per primer.

Classification of apricots

We obtained reproducible amplified DNA fragments among 33 apricot varieties of *P. armeniaca* and 2 species, using 18 primers (Fig. 1). We recorded the number of non-shared RAPDs for each pair of varieties (Table 4). 'Alpine plum' (*P. brigantina*) and 'Siberian apricot' (*P. sibirica*) had many bands which differed from *P. armeniaca*. On the contrary, no polymorphism were detected between 'Hajihaliloulu' and 'Hasanbay', whereas the pairs: 'Mochianzu' and 'Koushiuoumi', 'Heiwa' and 'Waseoumi', or 'Heiwa' and 'Li-zi-xing'

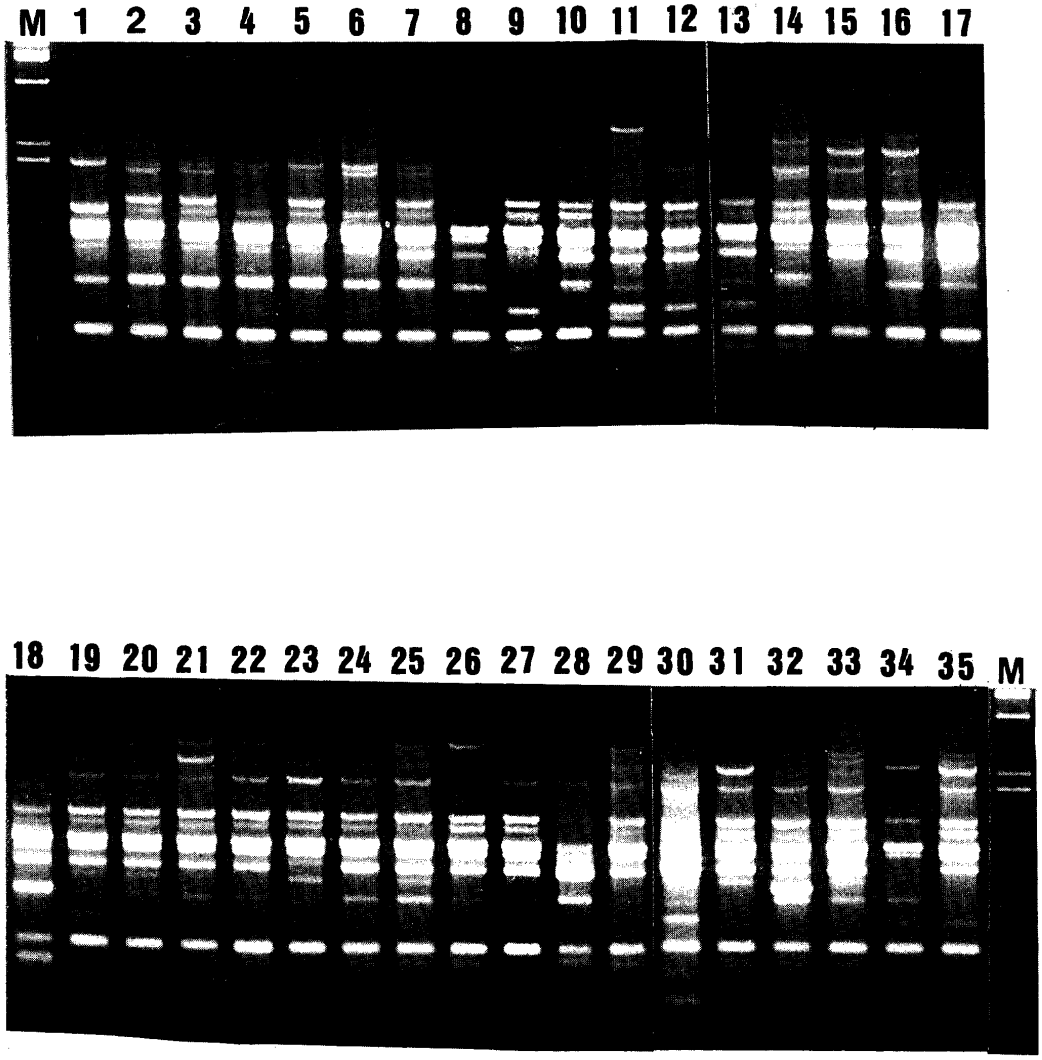

Fig. 1. RAPD patterns of 35 apricot varieties by OPC-11. M: Hind III digested lambda DNA. Lane 1; 'Siberian apricot', 2; 'Hajihaliloulu', 3; 'Hasanbay', 4; 'Tilton', 5; 'Goldcot', 6; 'Blenheim', 7; 'Harcot', 8; 'Early Orange', 9; 'Alexander', 10; 'Mei-tao-xing', 11; 'Hiroshima koanzu', 12; 'Mochianzu', 13; 'Kyoudai maru', 14; 'Mai-huang-zhun-xing', 15; 'Bai-xing', 16; 'Shimizugou', 17; 'Shinshiuoumi', 18; 'Ren-xing', 19; 'Koushiu oumi', 20; 'Niigata oumi', 21; 'Jinshirou', 22; 'Heiwa', 23; 'Yamagata 3', 24; 'Ogasawara', 25; 'Akita oumi', 26; 'Wase oumi', 27; 'Li-zi-xing', 28; 'Alpine plum', 29; 'Mameanzu', 30; 'Takanomanjiu', 31; 'Mikanmomo', 32; 'Aomorisan', 33; 'Nodokukuri', 34; Nepal apricot No. 85247, 35; Nepal apricot No. 85260.

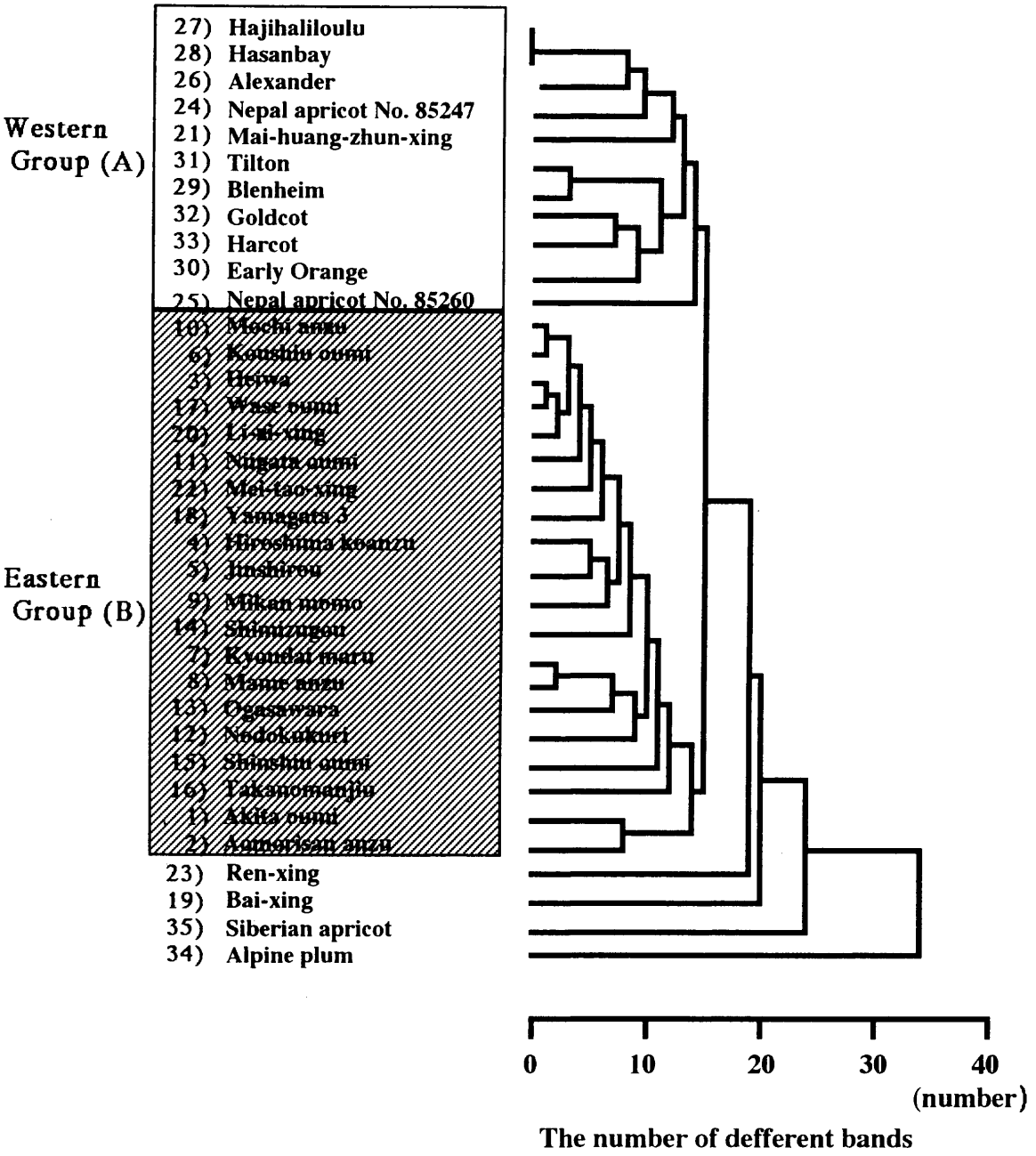

Fig. 2. Dendrogram of 35 apricot varieties based on the data from 58 RAPDs by cluster analysis using group average method. The numbers correspond to the code number in Table 1.

could be discriminated by one DNA fragment. 'Hajihaliloulu' and 'Hasanbay' resembled each other morphologically but were impossible to discriminate by any RAPD, suggesting that they may be synonyms.

We constructed the dendrogram by cluster analysis (Fig. 2) and scattergram by quantification method of the third type, plotting the first component to the X-axis and second to the Y-axis (Fig. 3). We categorized some varieties into one group, in which less than 15 bands were different from each other. Hence the dendrogram is divided into two large groups; one as "Western group" (A) which originated in Europe, Central Asia, and western China, and the other as "Eastern group" (B) which originated in eastern China and Japan. 'Alpine plum' (*P. brigantina*), 'Siberian apricot' (*P. sibirica*), 'Bai-Xing', and 'Ren-xing' did not belong to either groups, exhibiting large dissimilarities. 'Alpine plum' differed the most from the other apricots. DNA-wise and morphologically, 'Bai-Xing' and 'Ren-xing' differ from other cultivated species, 'Ren-xing' being considered as a natural hybrid between an apricot and a plum (King, 1940). With no knowledge about the variations among North Chinese varieties, we could not genetically characterize 'Bai-Xing'.

Kikuchi (1948) proposed that some morphological differences exist among European and Asian apricots, but they are not sufficient enough to separate them into different species. Contrarily, Bailey and Hough (1975) classified East Chinese apricots as *P. ansu,* because their characteristics such as flower color and flowering

Table 4. The number of non-shared 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	29	30	31	32	33	34	35
1	Akita oumi																																			
2	Aomorisan anzu	8																																		
3	Heiwa	13	13																																	
4	Hiroshima koanzu	16	14	5																																
5	Jinshirou	11	13	8	5																															
6	Koushiu oumi	14	14	3	4	5																														
7	Kyoudai maru	14	14	7	6	5	4																													
8	Mame-anzu	16	14	9	8	7	6	2																												
9	Mikanmomo	12	13	9	6	7	6	8	10																											
10	Mochi anzu	15	13	2	3	6	1	5	7	7																										
11	Niigata oumi	15	15	4	7	8	5	5	7	11	4																									
12	Nodokukuri	13	14	8	9	8	9	7	36	11	10	10																								
13	Ogasawara	9	9	12	11	10	9	7	7	11	10	10	12																							
14	Shimizugou	12	12	7	8	9	8	12	14	10	7	11	11	13																						
15	Shinshiu oumi	19	15	8	11	12	9	11	11	13	8	10	14	14	11																					
16	Takanomanjiu	14	14	11	8	11	10	10	12	12	9	9	15	9	14	15																				
17	Wase oumi	14	14	1	16	9	4	6	8	10	3	3	7	11	8	9	10																			
18	Yamagata 3	15	15	6	9	8	7	9	11	13	6	6	12	14	9	10	13	7																		
19	Bai-xing	23	21	18	19	20	21	21	21	19	20	20	20	26	21	14	25	19	18																	
20	Li-zi-xing	12	12	1	6	8	4	6	8	10	3	3	9	11	8	7	10	2	5	17																
21	Mai-huang-zhun-xing	20	18	11	10	15	12	14	16	12	11	13	15	21	14	13	18	12	15	19	12															
22	Mei-tao-xing	15	13	4	7	8	3	7	7	9	4	8	8	12	9	10	13	5	8	20	5	11														
23	Ren-xing	23	21	18	15	18	17	19	19	17	16	18	20	24	21	22	19	19	20	20	19	19	18													
24	Nepal apricot No. 85247	26	20	19	16	19	17	16	31	18	17	19	19	23	20	17	20	20	17	19	18	16	17	21												
25	Nepal apricot No. 85260	21	27	14	13	14	13	11	34	15	12	12	16	18	15	14	19	15	14	20	13	15	14	20	11											
26	Alexander	25	19	14	11	16	13	15	15	13	12	14	16	22	19	16	17	15	18	20	15	9	14	16	11	12										
27	Hajihaliloulu	25	19	14	11	16	13	15	15	13	12	16	16	22	17	12	17	15	14	14	15	11	12	16	9	14	8									
28	Hasanbay	25	19	14	11	16	13	15	15	13	12	16	16	22	17	12	17	15	14	14	15	11	12	16	9	14	8	0								
29	Blenheim	18	18	15	14	13	16	14	14	16	17	13	11	19	16	19	16	14	13	21	14	14	13	19	18	19	17	15	15							
30	Early Orange	24	18	15	14	17	16	16	16	16	15	15	17	23	18	11	18	16	17	13	14	12	15	15	12	13	9	11	11	14						
31	Tilton	17	17	12	13	12	13	13	13	15	14	12	12	20	15	16	17	13	10	18	11	11	10	16	15	16	14	12	12	3	11					
32	Goldcot	19	15	12	13	12	9	11	11	11	10	10	14	16	15	10	17	13	10	18	11	11	8	20	11	10	10	10	10	13	9	10				
33	Harcot	15	13	12	11	10	11	11	11	11	12	12	10	14	13	12	13	13	14	18	11	13	10	18	15	14	12	12	12	9	9	8	8			
34	Alpine plum	31	14	36	33	34	37	35	37	35	36	36	36	34	35	38	29	37	36	34	35	35	38	24	31	34	30	36	36	33	29	34	38	32		
35	Siberian apricot	25	15	38	25	24	27	25	23	25	26	26	26	36	23	28	23	29	24	30	27	27	26	24	17	20	26	26	26	23	23	32	22	20	26	

time differed. Yu (1979) supported Kikuchi's opinion that there are not enough differences between East Chinese apricots and European apricots to put them in separate species. Yoshida and Yamanishi (1988) also agreed with Kikuchi's consideration because members of the Eastern and Western groups hybridize very easily. As only a few Chinese apricots were available, it is difficult to discuss them any further. However, it was confirmed that there is no significant discontinuous variation of morphological and physiological characteristics between the Western (A) and Eastern (B) groups of apricots.

Nepalese apricots collected in the western Himalayas were classified into group A; 'Mai-huang-zhun-xing', Chinese cultivar, which is distributed from western to northern China was placed in this group. In group B, there were some cultivars which were presumed to be hybrids with mume (*Prunus mume* Sieb. et Zucc.) on the basis of morphological and ecological characteristics (Yoshida and Yamanishi, 1988). 'Ogasawara' and 'Akitaoumi', which seem to be hybrids between apricot and mume, clustered in this apricot group. Among the mume, there are many cultivars exhibiting apricot characteristics so they and the Japanese apricots may have co-evolved naturally.

The scattergram from the results of quantification method of the third type show a similar tendency to the cluster analysis, that is, materials were classified into two groups. The results by quantification method is not identical to the cluster analysis because the contribution of both components was approximately 39% which is not considered high. Nevertheless, we confirmed that the Chinese apricots have large variations; the range of positions in the scattergram of 'Bai-Xing', 'Ren-xing', 'Mei-tao-xing', 'Li-zi-xing', and 'Mai-huang-zhun-xing' is wide. Kikuchi (1948) described that traits of the apricots distributed in China is very diverse morphologically and placed the origin of apricots in eastern China. Considering the large variations in Chinese apricots, we strongly support the hypothesis that Chinese apricots are the ancestor of Japanese and European apricots.

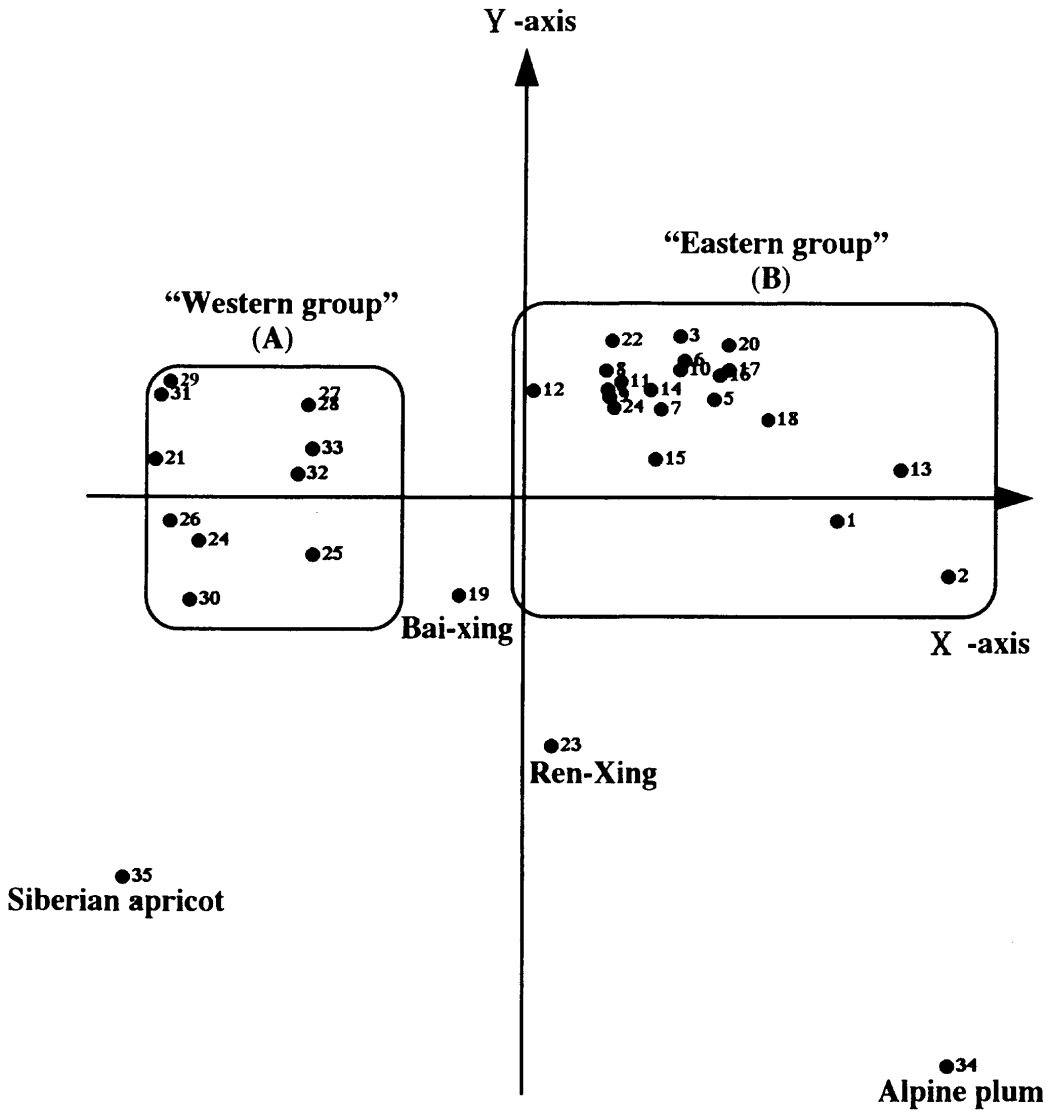

Fig. 3. The scattergram by quantification method of the third type. The numbers correspond to the code number in Table 1.

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RAPD 分析法によるアンズの系統分類

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摘　要

アンズの系統分類に RAPD 分析法を適用した．DNA 多型を効率的に検出するために，5 種類の代表的な品種を供試して，225 種類のオペロンプライマーについてスクリーニングを行い，各系統間で複数の DNA 多型を示す有効な 18 種類のプライマーを選抜した．次にこれらのプライマーを用いてアンズ 33 品種・系統と近縁の野生 2 種の分類を試み，検出された RAPDs をもとにクラスター分析と数量化理論第三類を用いてデータの解析を行った．その結果，本実験で供試したアンズ（*Prunus armeniaca*）の品種・系統は中国西部からヨーロッパにかけて分布する“西方品種群”（A）と中国東部，日本などに分布する“東方品種群”（B）の 2 群に大別された．

しかしながら，近縁野生種の *P. sibirica* と *P. brigantina*，中国の西部から北部に分布し，諸特性が不明である‘白杏’，およびスモモとアンズの自然交雑種とされる‘仁杏’はこれらの群に属さなかった．また，中国の品種は A 群および B 群の両方に属し，遺伝的変異が大きいことから日本アンズ，ヨーロッパアンズの祖先種である可能性が高いと推察した．