RAPD法によるボタンおよび雑種の品種比較

誌名	園藝學會雜誌
ISSN	00137626
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巻/号	66巻2号
掲載ページ	p. 393-400
発行年月	1997年9月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat

AgriKnowledge

J. Japan. Soc. Hort. Sci. 66 (2) : 393-400. 1997.

Comparative Study of Tree Peony (*Paeonia suffruticosa* Andr.) Cultivars and Hybrids by Random Amplified Polymorphic DNA (RAPD) Analysis

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Summary

Random amplified polymorphic DNA (RAPD) assay was performed to categorize or discriminate 14 tree peony (*Paeonia suffruticosa*) cultivars, a yellow tree peony (*P. lutea*), a Chinese peony (*P. lactiflora*) cultivar, and five interspecific cultivars. Forty decamer primers were screened; 11 of these primers produced 108 reproducible amplified DNA bands useful as polymorphic markers. Twenty one genotypes were discriminated with these markers. The similarity values among the genotypes were also calculated with these markers. The results showed that *P. suffruticosa* cultivars are clearly distinct species from *P. lactiflora* and *P. lutea*. Similarity values of yellow flowering hybrid cultivars, 'Kinkaku', 'Kinshi', 'Kinkoh', 'High Noon', and 'Oriental Gold' were between those of *P. suffruticosa* cultivars and *P. lutea*. Among *P. suffruticosa* cultivars, 'Shishigashira', which was introduced to Shimane Pref. from Shizuoka Pref. in the Edo-period, had lower similarity values with cultivars which originated from the Osaka Pref. during the Meiji era. *P. suffruticosa* cultivars with closely related parentage showed high similarity values (SVs) with both or either parent (s). Cultivar relationships based on SVs established by RAPD markers agreed partly with those based on morphological data, but not on the basis of petal anthocyanidins.

Introduction

Tree peony (*Paeonia suffruticosa* Andr.), which originated in China, was introduced into Japan in the 10 th century. Since then many Japanese cultivars were created especially during the late 17 th to the early 18 th century in the Edo period (1603-1867) (Hashida, 1990). In the Meiji era (1867-1912) *P. suffruticosa* cultivars were propagated in Ikeda City, Osaka Prefecture, and introduced into Yatsuka-cho (Daikon-jima Island), Shimane Pref., and Gosen City, Niigata Pref. Since the two prefectures have become major production areas of tree peonies in Japan and many cultivars were created from seedlings. Most of these Japanese cultivars originated from unknown parentages. In France, Henry and Lemoine during the early 20 th century hybridized *P. suffruticosa* cultivars with *P. lutea* Delav. ex Franch and obtained yellow cultivars (Wister and Wolfe, 1962). In the United States, Saunders created many American hybrid cultivars with the same combination of species but with different cultivars in the mid-twentieth century (Wister and Wolfe, 1962). Both French and American cultivars have been introduced into Japan and are much appreciated.

Previously Hamada et al. (1989) classified 26 tree peony cultivars and hybrids into 6 groups based on 18 morphological characteristics and constructed a dendrogram, illustrating cultivar relationships. Hosoki et al. (1991) also reported that 72 cultivars and hybrids could be classified into 4 groups based on flower anthocyanidins and chalcones. Although these classifications gave useful information for the breeding of tree peony cultivars and interspecific hybrids, genetic relationships among them were not clearly understood.

Recently more direct DNA analysis has been developed for objectively identifing cultivars. Ran-

Received; December 27, 1995. Accepted; November 25, 1996.

dom Amplified Polymorphic DNA (RAPD) analysis by polymerase chain reaction (PCR) (Williams et al., 1990) has been developed into a successful tool for identification of cultivars and clones which were barely distinguishable by their morphological characters (Mori et al., 1993; Sugiyama et al., 1995; Villordon and LaBonte, 1995; Wolff and Van-Rijin, 1993). Although many reports on cultivar identification and pursuit of origin using RAPD have been published in the field of fruit tree taxa (Aruna et al., 1995; Fabbri et al., 1995; Luo et al., 1995; Ozaki et al., 1995), those on ornamental trees are still limited; studies on cultivars and species relationships in rhododendron (Iqbal et al., 1995; Kobayashi et al., 1995), rose (Torres et al., 1993) or red maple (Krahi et al., 1993) have been reported using RAPD. In *Paeonia spp*, Sang et al. (1995) reported that several species could be classified using difference in nucleotide sequence of ribosomal DNA. However, discrimination among tree peony cultivars or hybrids has not been studied. Previously we examined isozyme band patterns of peroxidase and acid phosphatase in 20 cultivars but were not able to distinguish genetic differences (unpublished data).

The objective of the present experiment is to discriminate tree peony cultivars and hybrids, clarify their relationships using RAPD by PCR and to compare these results with the classifications based on morphology or petal pigments (Hamada et al., 1989; Hosoki et al., 1991).

Materials and Methods

Plant materials.

Fourteen Japanese tree peony cultivars (*P. suffruticosa*), a yellow tree peony (*P. lutea*), a Chinese peony cultivar (*P. lactiflora*), four yellow interspecific hybrid cultivars (*P. lutea* × *P. suffruticosa*), and 'Oriental Gold' ((*P. lactiflora* × (*P. lutea* × *P. suffruticosa*)) were studied (Table 1). Their petal color and pigments, and origin are also shown in Table 1. These materials were maintained in the field of Shimane University, Matsue, Shimane Pref.

DNA extraction.

Young immature leaves were collected from March to April in 1995 and stored at −20 °C before DNA extraction. Total DNA was extracted from sliced leaves based on Isoplant Manual of DNA extraction kit (Nippon Gene Co. Ltd., Toyama, Japan) ; 100 mg (fw) of leaves were cut into 1 mm strips and put into 300 μl extraction buffer containing sodium dodecyl sulfate (SDS). After adding RNase, the extract was mixed with 150 μl of benzyl chloride and then 150 μl of sodium acetate. After mixing and subsequent centrifugation, 700 μl of ethanol was added to the aqueous layer to precipitate the DNA. The crude solid DNA was rinsed with 70 % ethanol and redissolved with 50 μl TE buffer (pH 8.0) containing 10 mM Tris-HCl and lmM EDTA. The DNA in 50 μM TE buffer was re-extracted by the method of Stewart and Via (1993) by adding 200 μl 2 % cetyltrimethylammonium bromide (CTAB) containing 1.42 M NaCl, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 2 % polyvinylpirrolidone (PVP), 5 mM ascorbic acid and 4 mM diethyl dithio carbamic acid. The mixture was mixed with 200 μl of chloroform: isoamyl alcohol (24: l, v/v) and centrifuged at 12000 rpm for 5 min. An aqueous phase was separated and mixed with 200 μl isopropanol and centrifuged at 12000 rpm for 10 min. The precipitate (DNA) was rinsed with 1000 μl 70 % ethanol and centrifuged at 12000 rpm for 10 min. Finally, the precipitated DNA was dried with warm air and dissolved with 50 μl TE. This re-extraction procedure was repeated four times per each sample.

DNA amplification.

DNA amplification followed the method of Sugiyama et al. (1995) with a slight modification. Forty decamer primers (oligonucleotides) (kit A and B, Operon Technologies, Alameda, Calf., USA) were tested. Amplification reaction was conducted in 25 μl volumes, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dGTP, dCTP, and dTTP, 2 μM primer, 0.25 μl of 5 units/μl Taq DNA polymerase (Takara, Co Ltd., Ohtsu, Shiga, Japan) and about 0.7 μg genomic DNA dissolved in 1 μl TE. Each reaction mixture was overlaid with mineral oil to prevent evaporation during PCR. Reactions were performed in a Gene Amp RCR system 9600 (Perkin Elmer Cetus, Calif., USA) programed as follows: 3 min at 94 °C for initial strand separation, 50 cycles of 45 sec at 94 °C, 1 min at 40 °C and 1 min at 72 °C, followed by 5 min at 72 °C for final extension. PCR products were stored at 4 °C

Table 1. Petal clor, origin and history of 21 peony genotypes used for this study[z].

Code No.	Cultivar	Petal color[y]	Dominant anthocyanins or chalcone[x]	Origin and history
	Japanese tree peony cultivars (*P. suffruticosa*)			
1	'Kamadafuji' (鎌田藤)	Light purple (8603)	Pn	Created in Osaka before 1889.
2	'Gunpoden' (群芳殿)	Vivid reddish purple (8906)	Pn, Cy	Created in Osaka before 1934. A hybrid between 'Kamadafuji' and 'Hanadaijin'
3	'Hanadaijin' (花大臣)	Vivid red purple (9208)	Pn, Cy	Created in Osaka before 1910.
4	'Hanakisoi' (花競)	purple pink (9203)	Pg	Created in Osaka before 1910.
5	'Yatsukajishi' (八束獅子)	purple pink (9203)	Pg	Created by S. Kadowaki in Daikon-jima Island, Shimane in 1932. Seedling of 'Hanakisoi'.
6	'Renkaku' (連鶴)	Greenish white (3701)	−	Created in Osaka before 1889.
7	'Hakuunryu' (白雲竜)	White (-01)	−	Origin unknown
8	'Gessekai' (月世界)	White (-01)	−	Created in Osaka before 1934.
9	'Taiyoh' (太陽)	Vivid red (0107)	Pg	Created in Niigata before 1931.
10	'Shimadaijin' (島大臣)	Vivid red purple (9208)	Pn, Cy	Created by K. Ikeuchi in Daikon-jima Island, Shimane, in 1952.
11	'Kaoh' (花王)	Vivid purplish red (9707)	Pg	Created by C. Watanabe in Daikon-jima Island, Shimane, in 1931.
12	'Kurikawakoh' (栗皮紅)	Bright reddish purple (8905)	Pn, Cy	Origin unknown
13	'Nishikinotsuya' (錦の艶)	Bright purplish red (9706)	Pg	Created by J. Nagao in Niigata before 1931.
14	'Shishigashira' (獅子頭)	Purplish pink (9203)	Pg	Introduced from Shizuoka to Daikon-jima Island in Shimane during 1680s.
	Yellow tree peony (*P. lutea*)			
15	*P. lutea*	Vivid yellow (2507)	Ch	Discovered by P. Delavayi in Yunnan, China in 1883.

	Chinese peony cultivars (*P. lactiflora*)			
16	'Kakouden' (花香殿)	Yellowish white (2501)	–	Created before 1955.
	Yellow hybrid cultivars			
17	'Kinkaku' (金閣)	Vivid yellowish orange (1906) with orange fringe	Ch	'Souvenir de Maxim Cornu' (French name). Created by L. Henry in France in 1919. A hybrid between *P. lutea* and 'La Ville de St. Demis' (*P. suffruticosa* var. *spontanea*)
18	'Kinshi' (金鶏)	Vivid yellowish orange (1906) with orange base	Ch	'Chromatella' (French name). A sport of 'Kinkaku'. Found by E. Lemoine in France in 1928.
19	'Kinkoh' (金晃)	Vivid yellowish orange (1906)	Ch	'Alice Harding' (English name). Created by E. Lemoine in France in 1935. A hybrid between *P. lutea* and 'Yaso-okina' (*P. suffruticosa*)
20	'High noon' (ハイヌーン)	Vivid yellowish orange (1906)	Ch	Created by A. P. Saunders in USA in 1952. A hybrid between *P. lutea* and a Japanese tree peony cultivar.
21	'Oriental Gold' (オリエンタルゴールド)	Light yellow (2511)	Ch	Created by T. Itoh in 1958. A hybrid between 'Kakouden' (*P. lactiflora*) and 'Kinkoh' (*P. lutea* × *P. suffruticosa*)

[z] Referred to Adachi (1983), Hashida (1990), Haworth-Booth (1963), Hosoki et al. (1991) and Wister and Wolfe (1962).
[y] Referred to The Japan color standard for horticultural plants in parenthes
[x] Pn : peonidin, Cy : cyanidin, Pg : pelargonidin and Ch : chalcone.

before analysis. Amplified products were separated by gel electrophoresis in 1.5 % agarose S (Nippon Gene Co Ltd., Toyama, Japan) in TAE buffer containing 0.04 M Tris-acetete and 0.001 M EDTA and stained with ethidium bromide. The gels were photographed under UV light (365 nm) using Type 337 Polaroid film. The molecular sizes of amplified products were approximated, using DNA size marker, *λ/Hind* Ⅲ digest-*Φx174/Hinc* Ⅱ digest (Toyobo Co., Tokyo, Japan). All reactions were conducted three times, using different samples, which were collected at three different date, within 10 days from the same plant of each cultivar or species. Only reproducible bands were used for further analysis.

Data analysis

Similarity values for each genotype pair were estimated by the Nei's similarity index (Nei and Li, 1979) according to the following equation: Similarity value $= 2\ N_{ab}/\ (N_a + N_b)$; N_{ab} represents the number of counted amplification fragments with the same molecular weight shared between genotypes a and b, whereas N_a and N_b present the number of counted amplification fragments in genotypes a and b, respectively.

Table 2. List of the primers that produced RAPD polymorphisms among 21 peony genotypes.

Primer	Sequence	Number of bands		
		Total	Monomorphic	Polymorphic
OPA-07	GAAACGGGTG	15	4	11
OPA-08	GTGACGTAGG	12	2	10
OPA-09	GGGTAACGCC	12	1	11
OPA-18	AGGTGACCGT	11	4	7
OPA-19	CAAACGTCGG	13	1	12
OPA-07	GGTGACGCAG	11	0	11
OPA-08	GTCCACACGG	11	2	9
OPA-17	AGGGAACGAG	13	2	11
OPA-18	CCACAGCAGT	9	3	6
OPA-19	ACCCCCGAAG	12	1	11
OPA-20	GGACCCTTAC	12	3	9
Total		131	23	108

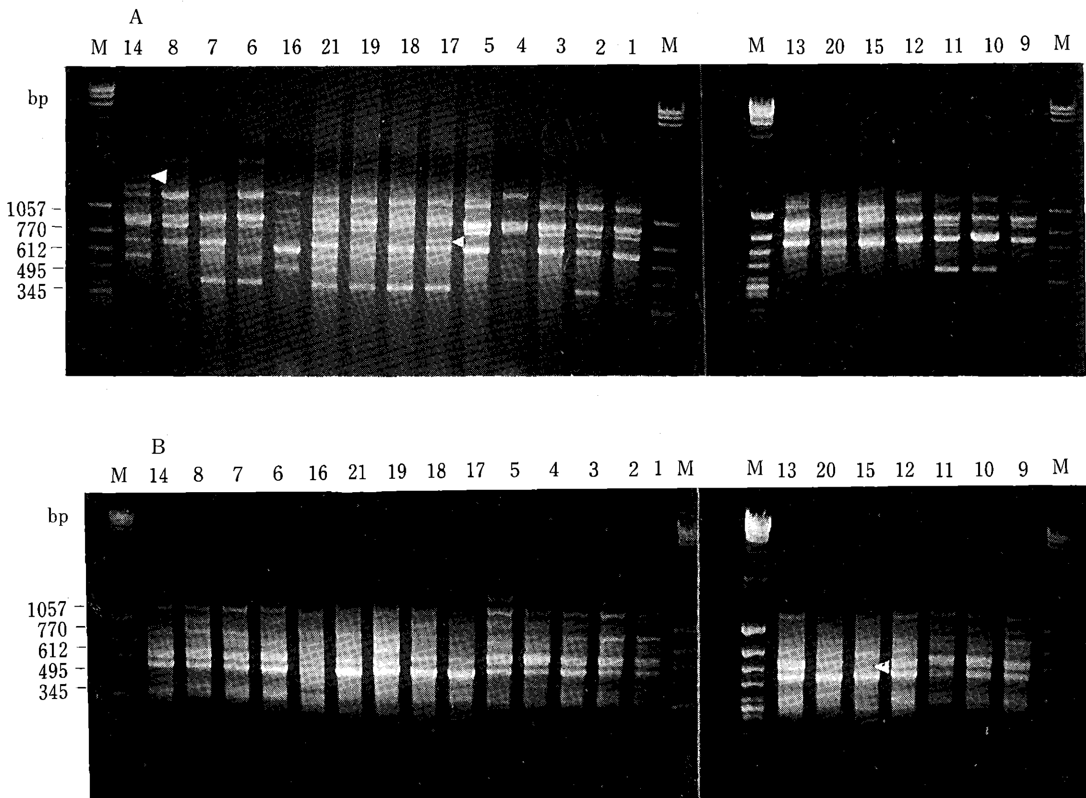

Fig. 1. RAPD pattern obtained from the 21 peony genotypes with the primer OPB-19 (A) and OPB-20 (B). Lane numbers correspond to code numbers given in Table 1. M: *λ/Hind* Ⅲ digest-*ϕx174/Hinc* Ⅱ digest DNA size marker showing base pairs (bp). Arrowheads in A indicate DNA bands present only in 14 or 17 and that in B indicates DNA band absent only in 15.

Results and Discussion

Out of 40 primers tested, eleven primers produced 9 to 15 DNA bands containing 7-12 polymorphic bands possible for data analysis in 21 genotypes. A total of 131 bands was produced from these 11 primers (Table 2), ranging in size from 340 to 1540 base pairs. Twenty eight primers produced only 2 to 3 polymorphic bands and one primer produced more than 20 bands, which made comparisons of genotypes difficult. These 29 primers were not used for data analysis.

Figure 1 shows an example of amplification pattern obtained with primer OPB-19 or OPB-20. Using 108 polymorphic bands of the total 131 bands obtained from the 11 primers, 21 genotypes were discriminated from each other because there were no genotype pairs in which all the bands were identical. Some bands were either present or absent in one genotype, which was considered genotype-specific markers.

Table 3 shows a similarity matrix calculated using 131 bands obtained from 21 genotypes. The similarity values (SVs) ranged from 0.653 between 'Yatsukajishi' (5) and *P. lutea* (15) to 0.971 between 'Kinkaku' (17) and (18) 'Kinshi' with an average of 0.800. Table 4 shows the mean of SVs calculated from Table 3. The mean of SVs between *P. suffruticosa* cultivars were 0.86 Approximate SVs between different species, *P. suffruticosa, P. lutea* or *P. lactiflora* , were 0.70, and those between species and yellow hybrids or 'Oriental Gold' (21) were 0.76. Thus, *P. lactiflora* or *P. lutea* is genetivally remote from Japanese *P. suffruticosa* cultivars whereas the yellow hybrid cultivars are of intermediate distance.

In a comparison among Japanese *P. suffruticosa* cultivars, the SVs ranged from 0.807 between (12) and (14) to 0.928 between (10) and (11), indicating that they are genetically close. This result is consistent with the history that the cultivars (1 to 13) which were created in Shimane and Niigata Prefs. but whose ancestor came from Osaka Pref. during the Meiji era (Hashida, 1990). The mean SV of 'Shishigashira' (14), which was introduced to Shimane Pref. from Shizuoka Pref. in the Edo-period (Adachi, 1983), was the lowest among the Japanese cultivars.

Tracing the parentage according to Hashida (1990) and relying on DNA data show that 'Hanakisoi' (4) and its seedling progeny, 'Yatsukajishi' (5) showed genetically close with a SV = 0.919; both are also close to 'Hanadaijin' (3), with SV = 0.917 and 0.920, respectively, so that 'Hanadaijin'

Table 3. Similarity matrix generated by using the Nei's estimate of similarity.

Code No.	Cultivar/Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	'Kamadafuji'	(鎌田藤)	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
2	'Gunpouden'	(群芳殿)	0.892	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3	'Hanadaijin'	(花大臣)	0.892	0.906	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
4	'Hanakisoi'	(花競)	0.854	0.881	0.917	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5	'Yatsukajishi'	(八束獅子)	0.855	0.896	0.920	0.919	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	'Renkaku'	(連鶴)	0.852	0.879	0.890	0.854	0.855	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7	'Hakuunryu'	(白雲竜)	0.819	0.847	0.835	0.798	0.798	0.879	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8	'Gessekai'	(月世界)	0.810	0.850	0.862	0.812	0.838	0.882	0.874	1.000	.	.	.	.	.	.	.	.	.	.	.	.	.
9	'Taiyoh'	(太陽)	0.838	0.866	0.890	0.877	0.892	0.850	0.866	0.857	1.000	.	.	.	.	.	.	.	.	.	.	.	.
10	'Shimadaijin'	(島大臣)	0.852	0.892	0.904	0.890	0.893	0.899	0.843	0.859	0.913	1.000	.	.	.	.	.	.	.	.	.	.	.
11	'Kaoh'	(花王)	0.831	0.871	0.859	0.857	0.847	0.879	0.859	0.850	0.890	0.928	1.000	.	.	.	.	.	.	.	.	.	.
12	'Kurikawakoh'	(栗皮紅)	0.826	0.842	0.854	0.817	0.817	0.874	0.842	0.893	0.861	0.874	0.889	1.000	.	.	.	.	.	.	.	.	.
13	'Nishikinotsuya'	(錦の艶)	0.862	0.854	0.865	0.840	0.829	0.874	0.854	0.821	0.873	0.874	0.865	0.860	1.000	.	.	.	.	.	.	.	.
14	'Shishigashira'	(獅子頭)	0.855	0.847	0.859	0.833	0.810	0.832	0.859	0.838	0.866	0.831	0.812	0.807	0.830	1.000	.	.	.	.	.	.	.
15	'*P. lutea*'	(キボタン)	0.720	0.701	0.688	0.671	0.653	0.701	0.740	0.702	0.662	0.680	0.662	0.671	0.723	0.701	1.000	.	.	.	.	.	.
16	'*P. lactiflora*'	(花香殿)	0.717	0.687	0.687	0.683	0.667	0.711	0.761	0.700	0.688	0.679	0.675	0.683	0.695	0.724	0.694	1.000	.	.	.	.	.
17	'Kinkaku'	(金閣)	0.783	0.788	0.800	0.750	0.736	0.798	0.835	0.766	0.780	0.783	0.776	0.760	0.795	0.776	0.766	0.724	1.000	.	.	.	.
18	'Kinshi'	(金鵄)	0.766	0.772	0.784	0.734	0.732	0.793	0.830	0.762	0.764	0.766	0.760	0.756	0.802	0.772	0.787	0.720	0.971	1.000	.	.	.
19	'Kinkoh'	(金晃)	0.734	0.763	0.763	0.725	0.711	0.761	0.809	0.765	0.731	0.769	0.740	0.747	0.782	0.740	0.752	0.735	0.890	0.908	1.000	.	.
20	'High Noon'	(ハイヌーン)	0.777	0.770	0.770	0.717	0.714	0.780	0.795	0.734	0.723	0.752	0.720	0.704	0.695	0.758	0.841	0.688	0.882	0.877	0.829	1.000	.
21	'Oriental Gold'	(ｵﾘｴﾝﾀﾙｺﾞｰﾙﾄﾞ)	0.747	0.753	0.764	0.750	0.725	0.762	0.798	0.743	0.733	0.770	0.753	0.737	0.760	0.753	0.753	0.760	0.854	0.860	0.928	0.817	1.000

Table 4. Mean of SVs of RAPD bands calculated from Table 3.

Contrast	Mean of SV
In *P. suffruticosa* (code No. 1-14)	0.86
P. suffruticosa (1-14) vs. *P. lutea* (15)	0.69
P. suffruticosa (1-14) vs. *P. lactiflora* (16)	0.70
P. suffruticosa (1-14) vs. yellow hybrids (17-20)	0.76
P. suffruticosa (1-14) vs. 'Oriental Gold' (21)	0.75
P. lutea (15) vs. *P. lactiflora* (16)	0.69
P. lutea (15) vs. yellow hybrids (17-20)	0.79
P. lutea (15) vs. 'Oriental Gold' (21)	0.75
P. lactiflora (16) vs. yellow hybrids (17-20)	0.72
P. lactiflora (16) vs. 'Oriental Gold' (21)	0.76
In yellow hybrids (17-20)	0.89
Yellow hybrids (17-20) vs. 'Oriental Gold' (21)	0.86

and 'Hanakisoi' are possibly derived from a common ancestor from Osaka Pref. Similarly, 'Shimadaijin' (10) and 'Kaoh' (11) whose SV = 0.928 may have been derived from a common origin in Shimane or Osaka Pref. 'Gunpouden' (2), a hybrid between 'Kamadafuji' (1) and 'Hanadaijin' (3) has a high SV = 0.906 with 'Hanadaijin'. 'Kinkaku' (17) and its sport, 'Kinshi' (18) are yellow hybrid cultivars with a SV value=0.971. 'Kinkaku' has petals with an orange fringe which differs from 'Kinshi' which has petals with an orange throat (Table 1). Such a difference in color position between two cultivars may be due to a gene modifier.

'Oriental Gold', a hybrid between 'Kakouden' (16) and 'Kinkoh' (19), exhibit a SV=0.928 with 'Kinkoh', whereas with 'Kakouden' the SV=0.760. This suggests that 'Oriental Gold' inherited a smaller genome from 'Kakouden' than that from 'Kinkoh', Further genetical and cytological study is necessary to verify this hypothesis.

As for the relationships between morphological classification and DNA analysis, yellow hybrid cultivars,which morphologically appear to be remotely related to Japanese *P. suffruticosa* cultivars (Hamada et al., 1989), also has low SVs (mean of 0.76). Among Japanese *P. suffruticosa* cultivars, those which are related (2 and 3, or 4 and 5) exhibit high SVs. 'Kamadafuji' (1) and 'Kurikawakoh' (12), which appear morphologically similar, have a SV = 0.826. Thus, further study is necessary to clarify anomaly between morphological and DNA classification.

As for classification based on petal pigments and DNA analysis, there is no clear correlation between them except for yellow hybrid cultivars. 'Hanadaijin' (3) and 'Kurikawakoh' (12) which contained the same anthocyanidins (Pn, Cy) (Table 1) has a SV = 0.874, whereas'Shimadaijin' (10) and 'Kaoh' (11) which contain different anthocyanidins (Pn plus Cy, and Pg, respectively) has a SV = 0.928. Yellow hybrid cultivars belong to the same group according to pigment and DNA analyses; the yellow pigment, chalcone was introduced from *P. lutea* (Haworth-Booth, 1963).

The white cultivars, 'Renkaku' (6), 'Hakuunryu' (7) and 'Gessekai' (8), are difficult to distinguish them visually from each other, Once the plants are mislavelled during propagation or shipping, they are indistinguishable to tree peony growers. Such a mixup could be resolved by checking their polymorphic DNA bands.

In conclusion, RAPD could be a useful tool to discriminate tree peony cultivars and hybrids whose appearances (petal color or morphology) are similar. Also in breeding of *P. suffruticosa* cultivars and hybrids, new cultivars with hybrid vigor can be obtained by selecting parents which would yield low SV by RAPD analysis.

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RAPD法によるボタンおよび雑種の品種比較

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摘　要

RAPD法により14のボタン（*Paeonia suffruticosa*）品種，キボタン（*P. lutea*）およびシャクヤク（*P. lactiflora*）品種および種間交雑5品種の識別を試みた．40種の10 merのプライマーを試験した結果，11種で多型マーカーとして有効な108本のDNAバンドを増幅した．これらのマーカーにより21種・品種が区別でき相互間の類似値が求められた．

その結果，ボタン品種はシャクヤク品種やキボタンと明らかに区別できた．またボタンとキボタンとの種間交雑品種である'金閣'，'金鵄'，'金晃'およびシャクヤク×'金晃'の'オリエンタルゴールド'の類似値はボタン品種との中間の値を示した．ボタン品種の内，江戸時代に静岡県から島根県に導入された'獅子頭'は，明治時代に大阪府を起源とする他の品種と比べて類似値が低かった．親子関係にあるボタン品種は両親または片親と高い類似値を示した．RAPDによる品種の類似関係は形態による分類と部分的に一致したが，花弁のアントシアニジンによる分類とは一致しなかった．