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Randomly Amplified Polymorphic DNA Analyses of *Pestalotiopsis theae* Isolated from Sweet Persimon

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재배되는 단감나무로 부터 분리한 *Pestalotiopsis theae*의 RAPD 기법을 이용한 유전특성의 비교분석

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ABSTRACT: In this study, we evaluated the genetic relationships of fourty seven *Pestalotiopsis theae* isolates collected from diseased sweet persimon in various places in southern part of Korea using RAPD (Randomly Amplified Polymorphic DNAs) method. As a result of the amplification, eight primers showed total of 86 bands ranging from 0.3 Kb to 3.2 Kb. Among those 86 bands, 84 polymorphic bands were used for bionominal matrix code (0, 1), and UPGMA dendrogram analysis. Similarities among the compared isolates ranged from below 60% to more than 95%. Most of the compared isolates showed 50~80% similarities. The number of isolate pairs which showed more than 80% similarity were 248. The number of isolate pairs which showed 50~80% similarity were 789, and the number of isolate pairs which showed below 50% similarity were 21. Isolate SP-21 (No. 9) showed below 50% similarity with all the isolates compared. At 50% similarity level, all the isolates compared, except isolate SP-21 (No. 9), were included in one big group. At 65% similarity level, all the isolates compared, except isolate SP-21 (No. 9), were divided into three different groups. At 75% similarity level, all the isolates compared, except isolates SP-47 (No. 23) and SP-21 (No. 9), were divided into six different groups.

KEYWORDS: *Pestalotiopsis theae*, RAPD method

As the cultivation area of sweet persimon (*Diospyros kaki* Thumb.) has increased in recent years, it became one of three major fruit crops in Korea. Cultivars of sweet persimon include Seo-cheon, Bu-you, Cha-rang, Seo-chon-cho-saeng, and Dae-ahn-dan-gam. Cultivation areas increased every year since 1980 when the cultivation area was 2,700 ha. High consumer demands for sweet persimon due to their characteristic taste and high economic profits made farmers to expand the cultivation area into 13,200 ha and 25,000 ha in 1994 and 1995, respectively. Most of cultivars planted in southern part of Korea is Bu-you (81.6%) (Kim 1992, Chang *et al.*, 1996, Chang *et al.*, 1997). This monoculture and increase of cultivation area resulted in serious disease problem, especially leaf blight caused by *Pestalotiopsis* spp. Leaf blight on sweet persimon occurred on cultivar Bu-you in 1990, and spread to other cultivars in Kimhae, Kyungnam and Kyungju, Kyungbuk, areas in Korea.

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Pathogenic isolates were collected from diseased sweet persimon leaves and later identified as *P. theae* (Guba 1929, Guba 1932, Ouekket and Seifert 1993, Kim *et al.*, 1997, Chang *et al.*, 1996, Chang *et al.*, 1997). This pathogen has wide host ranges, and causes deterence of furit formation and flowering problem next season (Kim *et al.*, 1997).

Direct analysis of DNA polymorphism is a more general approach to establishing genetic variation in organisms. Molecular methods involving the use of polymerase chain reation (PCR) have been recently described to resolve genetic variation between strains within the same species. The analysis of random amplified polymorphic DNA (RAPD) was proposed for discrimination between isolates of *Pestalotiopsis* species (Choi *et al.*, 1997, Choi *et al.*, 1997, Kim *et al.*, 1997, Kim *et al.*, 1997). In this study, therfore, RAPD method was used to elucidate the relatiohships among the *P. theae* isolates and to differentiate *Pestalotiopsis theae*, a leaf blight pathogen of sweet persimon (Guthrie *et al.*, 1992, Hartung *et al.*, 1993, Ouekket and Seifert 1993, Smith *et al.*, 1994, Williams *et al.*, 1990, Kim *et al.*, 1997).

Materials and Methods

Culture of *P. theae*

A collection of isolates of *P. theae* was made by direct isolation from diseased sweet persimon in various places in southern parts of Korea (Table 1). Culture of the isolates were made on a sterile filter paper placed on PDA for 10~15 days at 24°C.

DNA extraction

For large-scale preparations of pure DNA, fungal isolates were cultivated at 24°C for 10~15 days on a filter paper in PDA. The mycelium was harvested by scaping the surface of filter paper, lyophilized and ground with pestle in a mortar containing liquid nitrogen. A 1.5 m*l* microtube was filled halfway up the conical portion with ground lyophilized mycelium, and 750 m*l* of extraction buffer (pH 8.0) (500 m*l* NaCl, 100 mM Tris-Hcl, 50 mM EDTA, 1.25% SDS) was added. The extraction then was performed by following Rogers and Bendich's procedure (Rogers and Bendich, 1988). Finally, DNA was dissolved in TE buffer (pH 8.0) (10 mM Tris-HCl, and 1 mM EDTA), with a final concentration of 2 $\mu g/\mu l$, and stored at 4°C until use.

PCR reaction

For PCR amplification, 10 ng of genomic DNA was added into 20 mM dNTP and 0.5 μM of random primers (Operon Tech., Inc., USA) (Table 2). PCR reaction was performed with modified methods of Williams *et al.*, (1990) and Crowhurst *et al.*, (1991). One unit of Taq polymerase (Dynazyme^{TM}) was then mixed and final volum of 20 μl was used for the reaction. Reaction condition was consisted of 5 min at 95°C (preheating), 1 min at 94°C, 1 min at 35°C, and 2 min at 72°C for 35 cycles followed by storage at 4°C. Ten or fifteen μl of the amplified reaction products were run in 1.5% agarose gel containing ethidium bromide (0.5 $\mu g/ml$) in 0.5×TBE buffer for two hours and the results were viewed on UV transilluminator.

Data analysis

A computer program, NTSYS-pc Version 1.80 (Rohlf, 1993), was used for analysis of the electrophoretic data. Simple matching coefficients (Ssm) for each pair of isolates were calculated as described by Sneath and Sokal (1973) by formular: $S_{sm}=m/(m+u)$ where 'm' is the number of bands found in common between two isolates, and 'u' is the total number of bands unique to each sample. The matrix of similarity coefficients was then subjected to a clustering algorithm, the un-

Table 1. Isolates of *Pestalotiopsis theae* used for randomly amplified polymorphic DNA analysis

No.	Isolate	Sources	Locations	Year
1	SP-1	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
2	SP-4	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
3	SP-6	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
4	SP-12	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
5	SP-13	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
6	SP-17	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
7	SP-18	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
8	SP-20	Sweet persimmon	Kyongju, Kyongsangbuk-do	1996
9	SP-21	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
10	SP-24	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
11	SP-25	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
12	SP-26	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
13	SP-27	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
14	SP-28	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
15	SP-33	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
16	SP-34	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
17	SP-35	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
18	SP-38	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
19	SP-39	Sweet persimmon	Kimhae, Kyongsangnam-do	1996
20	SP-42	Sweet persimmon	Miryang, Kyongsangnam-do	1996
21	SP-45	Sweet persimmon	Miryang, Kyongsangnam-do	1996
22	SP-46	Sweet persimmon	Miryang, Kyongsangnam-do	1996
23	SP-47	Sweet persimmon	Miryang, Kyongsangnam-do	1996
24	SP-48	Sweet persimmon	Miryang, Kyongsangnam-do	1996
25	SP-49	Sweet persimmon	Miryang, Kyongsangnam-do	1996
26	SP-50	Sweet persimmon	Miryang, Kyongsangnam-do	1996
27	SP-57	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
28	SP-59	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
29	SP-61	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
30	SP-62	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
31	SP-66	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
32	SP-68	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
33	SP-69	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
34	SP-73	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
35	SP-77	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
36	SP-82	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
37	SP-85	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
38	SP-86	Sweet persimmon	Ankang, Kyongju, Kyongbuk	1996
39	97-KH-2	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
40	97-KH-3	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
41	97-KH-5	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
42	97-KH-6	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
43	97-KH-8	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
44	97-KH-10	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
45	97-KH-11	Sweet persimmon	Kimhae, Kyongsangnam-do	1997
46	97-KJ-2	Sweet persimmon	Kyongju, Kyongsangbuk-do	1997
47	97-KJ-5	Sweet persimmon	Kyongju, Kyongsangbuk-do	1997

weighted pair-group method with arithmatic average (UPGMA), that was then used to generate a dendrogram (Romesburg, 1984; Sneath and Sokal, 1973).

Results and Discussion

Genetic analyse of *Pestalotopsis theae* isolates collected from diseased sweet persimon fruits

Table 2. List of primer (10-mer) and their base sequences used for RAPD analysis

Primer No.	Base sequences	No. of bands
OPA-8	GAG ACG TAG G	12(12)[a]
OPA-12	TCG GCG ATA G	13(13)
OPA-14	TCT CGA ACC C	13(12)
OPA-15	TTC CGA ACC C	8(7)
OPA-16	AGC CAG CGA A	12(12)
OPA-17	CAC CGC TTC T	16(16)
OPA-18	AGG TGA CCG T	7(7)
OPA-19	CAA ACG TCG G	5(5)
Total		86(84)

[a]The number in the parentheses are the numbers of polymorphic bands for each.

and leaves of various locations were conducted with RAPD method. As a result, total of eighty six various sizes of bands (Fig. 1) were observed after the extracted genomic DNA was amplified with eight OPA primers (Table 2). Bands with clear differences were used for the anlysis of polymorphism among the compared *Pestalotiopsis theae* isolates. Polymerase chain reaction produced total of eighty six bands. Among these bands, 84 polymorphic bands were observed. Sizes of amplified genomic DNA ranged from 0.3 Kb to 3.2 Kb. Bionomical matrix code (1; presence of band, 0; absence of band) was constructed with 86 bands reacted with 8 primers. Based on the bionominal matrix code constructed, similarity matrix was made (Table 3) with NTSYS-PC (Rohlf, 1990) and then UPGMA dendrogram was constructed (Fig. 2).

Similarities among the compared isolates ranged from below 60% to more than 95%. Most of the compared isolates showed 50~80% similarities. The number of isolate pairs which showed more than 80% similarity were 248. The number of isolate pairs which showed 50~80% similarity were 789, and the number of isolate pairs which showed below 50% similarity were 21. Isolate SP-21 (No. 9) showed below 50% similarity with all the isolates compared.

At 50% similarity level, all the isolates compared, except isolate SP-21 (No. 9), were included in one big group. At 65% similarity level, all the isolates compared, except isolate SP-21 (No. 9), were divided into three different groups. At 75% similarity level, all the isolates compared, except isolates SP-47 (No. 23) and SP-21 (No. 9), were divided into six different groups.

All the primers used formed bands ranging from 1.0 Kb to 1.2 Kb in molecular weight. OPA primers 12, 14, 16 and 17 formed similar bands at 0.5 Kb. OPA-8 primer did not form any band at 1Kb with isolates 97-KH-2 (No. 39), 97-KH-5 (No. 41), 97-KH-6 (No. 42), 97-KH-8 (No. 43), 97-KH-10 (No. 44), and 97-KJ-2 (No. 46). Instead, they formed bands at 0.5 Kb with isolates SP-6 (No. 3), SP-20 (No. 8), SP-25 (No. 11), 97-KH-2 (No. 39), 97-KH-5 (No. 41), 97-KH-6 (No. 42) and 97-KH-10. OPA-12 primer did not form bands at 0.5 Kb with isolates SP-21 (No. 9) and SP-68 (No. 32). OPA-15 primer formed bands at 0.5 Kb with isolates SP-28 (No. 14), SP-33 (No. 15), SP-34 (No. 16), SP-35 (No. 17), SP-38 (No. 18) and SP-39 (No. 19). This last result was obtained from isolates collected from Jinhae, Kyungsangnamdo region, and close relationship was thus verified based on this result. In all primers, isolate SP-21(No. 9) showed different band patterns.

P. theae isolates collected from Kyungju, Kyungbuk showed relatively high affinity with isolates collected from Kimhae, Kyungsangdamdo. Also, isolates SP-49 (No. 25) and SP-50 (No. 26) collected from Milyang, Kyungnam showed high similarity with those form Kyungju, Kyungbuk. As shown in this study, there was no big difference in similarity among the isolates collected from Kyungbuk and Kyungnam, and no uniform patterns of bands were observed under the RAPD conditions used.

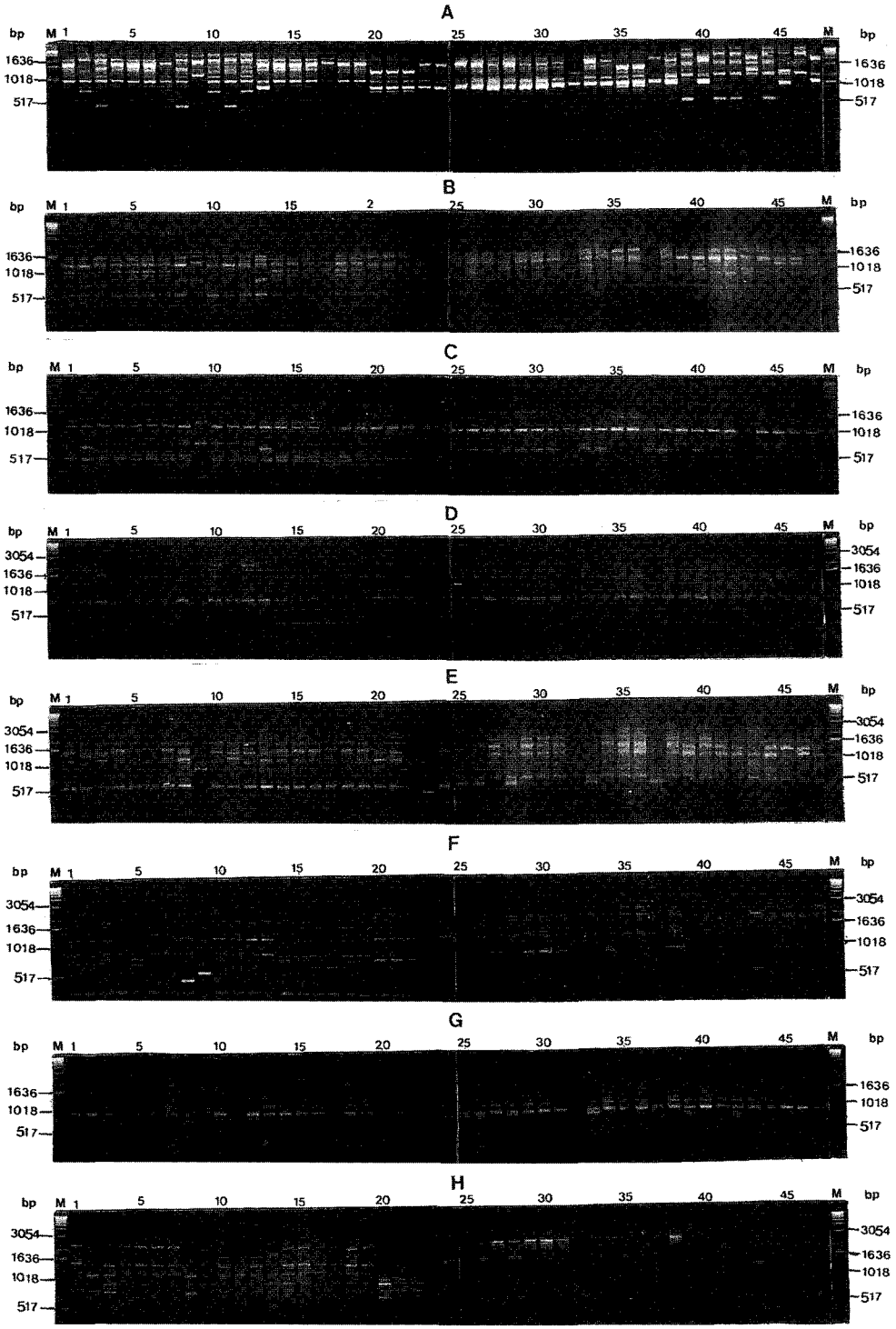

Fig. 1. PCR amplified DNA fragments using the primers listed in Table 2. A, B, C, D, E, F, G and H indicate the primer number OPA-8, -12, -14, -15, -16, -17, -18 and -19, respectively. The numbers on top of the lane indicate the *Pestalotiopsis theae* isolates shown in Table 1.

Table 3. Similarity matrix based on the number of shared bands by the compared *Pestaloiopsis theae* on 1.5% agarose gel in RAPD analysis[a]

	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	42	43	44	45	46
2	0.709																																													
3	0.790	0.790																																												
4	0.965	0.709	0.790																																											
5	0.906	0.720	0.779	0.930																																										
6	0.883	0.651	0.755	0.906	0.895																																								
7	0.860	0.651	0.686	0.860	0.848	0.895																																							
8	0.651	0.674	0.755	0.651	0.639	0.639	0.593																																						
9	0.488	0.534	0.569	0.488	0.500	0.453	0.430	0.546																																					
10	0.662	0.755	0.813	0.662	0.651	0.627	0.581	0.720	0.558																																				
11	0.639	0.709	0.744	0.639	0.627	0.604	0.534	0.767	0.558	0.732																																			
12	0.674	0.744	0.802	0.674	0.662	0.639	0.593	0.709	0.546	0.930	0.720																																		
13	0.837	0.744	0.779	0.813	0.802	0.802	0.779	0.639	0.523	0.651	0.627	0.686																																	
14	0.930	0.674	0.755	0.906	0.895	0.895	0.872	0.639	0.476	0.627	0.627	0.662	0.872																																
15	0.918	0.662	0.744	0.895	0.883	0.906	0.883	0.651	0.465	0.616	0.616	0.651	0.860	0.976																															
16	0.906	0.651	0.732	0.883	0.872	0.895	0.872	0.639	0.476	0.627	0.627	0.662	0.848	0.965	0.976																														
17	0.872	0.639	0.697	0.872	0.860	0.813	0.767	0.604	0.441	0.616	0.616	0.627	0.744	0.860	0.848	0.860																														
18	0.918	0.639	0.720	0.895	0.860	0.883	0.860	0.627	0.465	0.593	0.593	0.627	0.837	0.953	0.965	0.953	0.848																													
19	0.906	0.651	0.732	0.906	0.872	0.895	0.872	0.639	0.476	0.604	0.604	0.616	0.825	0.941	0.953	0.941	0.837	0.953																												
20	0.825	0.709	0.767	0.848	0.813	0.790	0.790	0.720	0.511	0.662	0.662	0.674	0.767	0.813	0.802	0.790	0.755	0.802	0.837																											
21	0.813	0.720	0.755	0.813	0.779	0.755	0.779	0.709	0.546	0.651	0.674	0.662	0.779	0.802	0.790	0.779	0.720	0.790	0.825	0.953																									
22	0.790	0.697	0.755	0.767	0.732	0.756	0.732	0.709	0.523	0.651	0.697	0.662	0.755	0.779	0.767	0.755	0.697	0.767	0.779	0.906	0.941																								
23	0.744	0.651	0.639	0.744	0.709	0.732	0.639	0.593	0.500	0.534	0.604	0.523	0.662	0.709	0.697	0.686	0.767	0.697	0.709	0.651	0.662	0.686																							
24	0.825	0.662	0.697	0.825	0.767	0.790	0.744	0.627	0.465	0.569	0.616	0.581	0.744	0.813	0.825	0.813	0.848	0.825	0.813	0.709	0.720	0.697	0.790																						
25	0.755	0.616	0.651	0.755	0.697	0.744	0.697	0.604	0.558	0.523	0.569	0.558	0.720	0.744	0.732	0.720	0.686	0.732	0.744	0.686	0.720	0.697	0.767	0.755																					
26	0.779	0.569	0.627	0.779	0.744	0.790	0.767	0.581	0.511	0.500	0.500	0.534	0.720	0.790	0.802	0.790	0.709	0.802	0.813	0.732	0.720	0.674	0.697	0.732	0.918																				
27	0.813	0.674	0.686	0.813	0.755	0.755	0.732	0.546	0.476	0.558	0.534	0.546	0.779	0.779	0.767	0.755	0.720	0.767	0.802	0.720	0.709	0.662	0.686	0.720	0.837	0.837																		
28	0.825	0.593	0.651	0.825	0.790	0.837	0.813	0.534	0.511	0.523	0.476	0.534	0.744	0.813	0.825	0.813	0.732	0.825	0.837	0.709	0.697	0.651	0.697	0.755	0.872	0.941	0.860																
29	0.813	0.674	0.686	0.813	0.779	0.779	0.755	0.546	0.500	0.558	0.534	0.546	0.802	0.802	0.790	0.779	0.720	0.790	0.825	0.744	0.732	0.686	0.686	0.720	0.837	0.860	0.965	0.883														
30	0.813	0.674	0.686	0.813	0.779	0.779	0.755	0.546	0.500	0.558	0.534	0.546	0.802	0.802	0.790	0.779	0.744	0.790	0.825	0.744	0.732	0.686	0.686	0.720	0.813	0.837	0.941	0.860	0.965													
31	0.837	0.674	0.686	0.813	0.755	0.755	0.732	0.546	0.476	0.581	0.534	0.569	0.802	0.802	0.790	0.779	0.767	0.790	0.779	0.697	0.686	0.662	0.709	0.744	0.813	0.813	0.918	0.837	0.918	0.918												
32	0.627	0.604	0.569	0.651	0.639	0.616	0.569	0.523	0.569	0.511	0.558	0.500	0.546	0.593	0.581	0.569	0.627	0.581	0.593	0.604	0.616	0.639	0.732	0.651	0.674	0.604	0.593	0.604	0.593	0.593	0.616											
33	0.755	0.616	0.674	0.755	0.720	0.767	0.720	0.581	0.558	0.569	0.523	0.534	0.697	0.74	0.755	0.744	0.662	0.755	0.790	0.709	0.720	0.697	0.744	0.686	0.872	0.895	0.813	0.872	0.837	0.837	0.813	0.651										
34	0.825	0.662	0.697	0.825	0.790	0.767	0.744	0.581	0.511	0.593	0.569	0.558	0.790	0.813	0.802	0.790	0.732	0.802	0.837	0.779	0.767	0.720	0.720	0.709	0.802	0.825	0.906	0.825	0.930	0.930	0.906	0.627	0.872									
35	0.779	0.662	0.720	0.779	0.744	0.744	0.744	0.604	0.558	0.639	0.546	0.604	0.767	0.767	0.755	0.744	0.686	0.755	0.790	0.802	0.813	0.767	0.674	0.662	0.779	0.802	0.813	0.802	0.837	0.837	0.813	0.651	0.825	0.848								
36	0.779	0.709	0.744	0.755	0.720	0.697	0.697	0.604	0.558	0.639	0.546	0.604	0.744	0.744	0.732	0.720	0.686	0.732	0.744	0.755	0.767	0.744	0.674	0.662	0.779	0.779	0.813	0.779	0.813	0.813	0.837	0.651	0.802	0.802	0.941							
37	0.569	0.569	0.558	0.593	0.627	0.604	0.558	0.488	0.511	0.500	0.476	0.465	0.511	0.558	0.546	0.534	0.593	0.546	0.581	0.569	0.558	0.558	0.674	0.569	0.662	0.662	0.604	0.662	0.627	0.651	0.604	0.767	0.732	0.662	0.616	0.616						
38	0.779	0.686	0.697	0.779	0.720	0.720	0.697	0.534	0.511	0.616	0.569	0.581	0.744	0.744	0.732	0.720	0.709	0.732	0.767	0.709	0.720	0.674	0.674	0.709	0.802	0.779	0.883	0.802	0.883	0.906	0.883	0.627	0.825	0.872	0.825	0.825	0.616					
39	0.744	0.674	0.755	0.744	0.709	0.709	0.709	0.686	0.546	0.651	0.604	0.616	0.686	0.732	0.720	0.709	0.651	0.720	0.755	0.767	0.779	0.755	0.639	0.651	0.767	0.767	0.709	0.744	0.732	0.732	0.709	0.662	0.813	0.767	0.813	0.790	0.627	0.720				
40	0.767	0.674	0.686	0.767	0.709	0.732	0.709	0.616	0.546	0.581	0.581	0.546	0.755	0.755	0.744	0.732	0.697	0.744	0.779	0.697	0.709	0.686	0.732	0.697	0.860	0.813	0.848	0.790	0.848	0.848	0.848	0.686	0.860	0.860	0.837	0.837	0.627	0.837	0.825			
41	0.732	0.709	0.790	0.732	0.674	0.674	0.674	0.697	0.558	0.686	0.616	0.651	0.674	0.674	0.662	0.651	0.616	0.662	0.697	0.732	0.744	0.720	0.604	0.616	0.732	0.709	0.720	0.709	0.720	0.720	0.720	0.651	0.779	0.732	0.825	0.825	0.593	0.732	0.930	0.837		
42	0.686	0.662	0.744	0.662	0.604	0.604	0.604	0.651	0.534	0.616	0.569	0.627	0.651	0.651	0.639	0.627	0.616	0.662	0.651	0.662	0.674	0.674	0.627	0.616	0.686	0.662	0.674	0.662	0.674	0.697	0.697	0.581	0.709	0.662	0.755	0.779	0.546	0.686	0.837	0.744	0.895	
43	0.813	0.674	0.755	0.813	0.755	0.755	0.709	0.639	0.593	0.651	0.604	0.616	0.755	0.779	0.767	0.755	0.720	0.767	0.802	0.790	0.779	0.755	0.709	0.674	0.790	0.790	0.802	0.767	0.802	0.802	0.802	0.709	0.860	0.837	0.837	0.837	0.674	0.790	0.825	0.872	0.837	0.744
44	0.686	0.686	0.767	0.709	0.674	0.674	0.627	0.767	0.534	0.709	0.662	0.674	0.627	0.651	0.662	0.651	0.639	0.662	0.697	0.732	0.720	0.697	0.627	0.639	0.709	0.709	0.674	0.686	0.674	0.674	0.674	0.674	0.779	0.709	0.779	0.779	0.639	0.686	0.883	0.790	0.918	0.825	0.813	
45	0.802	0.732	0.790	0.825	0.790	0.767	0.697	0.674	0.534	0.709	0.662	0.697	0.697	0.744	0.732	0.720	0.732	0.732	0.767	0.779	0.744	0.720	0.697	0.662	0.755	0.755	0.813	0.779	0.813	0.813	0.790	0.697	0.802	0.802	0.825	0.825	0.662	0.802	0.790	0.813	0.825	0.755	0.860	0.848
46	0.744	0.697	0.755	0.767	0.732	0.709	0.639	0.709	0.569	0.651	0.674	0.639	0.662	0.709	0.697	0.686	0.720	0.697	0.732	0.744	0.732	0.709	0.732	0.720	0.813	0.767	0.779	0.767	0.779	0.802	0.755	0.709	0.790	0.790	0.790	0.790	0.674	0.790	0.825	0.802	0.813	0.790	0.802	0.837	0.883
47	0.755	0.639	0.674	0.755	0.697	0.720	0.674	0.581	0.534	0.546	0.546	0.558	0.790	0.720	0.709	0.697	0.732	0.709	0.697	0.686	0.674	0.674	0.744	0.732	0.802	0.755	0.790	0.779	0.790	0.813	0.813	0.674	0.732	0.755	0.755	0.779	0.616	0.779	0.697	0.790	0.686	0.686	0.767	0.662	0.755	0.790

[a]Dara matrix was made by scoring the presence or absence of the bands as 1 and 0, respectively. Similarity coefficients between two isolates were then calculated with modified formular of Sneath and Sokal (1973).

[b]Isolate numbers.

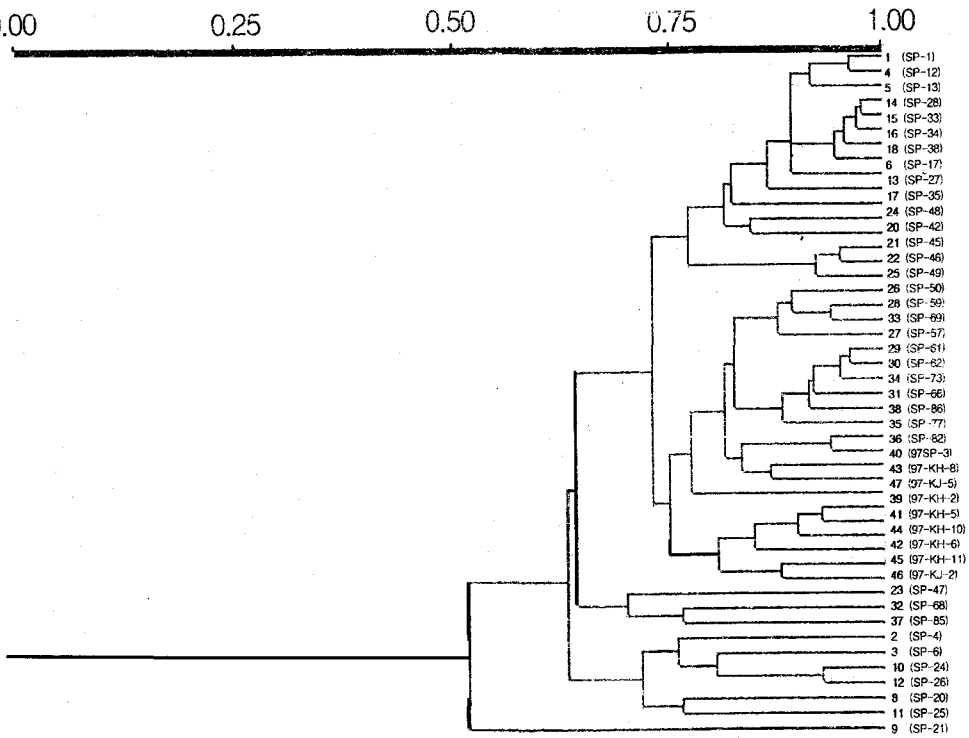

Fig. 2. UPGMA dendrogram showing the relationship among the *Pestalotiopsis theae* based on the bands on 1.5% agarose gel in RAPD.

적 요

단감이 경제과수로 점차 부각되면서 재배면적이 증가하는 반면 단일 품종으로 편중됨에 따라 주요 재배지를 중심으로 그간 문제시 되지 않았던 병해가 발생하여 이로 인해 단감의 품질과 농가 소득에 많은 손해를 초래하고 있다. 이중 중요 병해는 *Pestalotiopsis theae*에 의한 단감나무 둥근갈색무늬병을 들 수 있고, 아직까지 국내에서는 *Pestalotiopsis theae*에 대한 구체적인 연구가 수행되지 않고 있는 실정이며, 본 연구는 이들 병원균에 대한 기초정보를 밝히기 위하여 수행되었다. Random amplified polymorphic DNA(RAPD)를 사용하여 *P. theae*의 유전적 유연관계를 분석하였다. P-28 (No. 14)과 SP-33 (No. 15)은 0.976으로 97%의 가장 높은 유사성을 나타났으며 SP-18 (No. 7)과 SP-21 (No. 9)은 0.430으로 가장 낮은 유사성을 보였다. 서로 비교한 균주들 간의 유사성은 60% 이하로 부터 95% 이상인 것도 있었으며, 대다수의 균주들은 50~80%의 유사성을 나타내었다. SP-21 (No. 9)은 고립된 한 집단으로 나타났으며 비교한 모든 균주와 60% 이하의 낮은 유사성을 나타내었다.

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