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Analyses of Genetic Relationships of *Rhizoctonia solani* Isolates from Various Crop Species and Rapid Identification of Anastomosis Groups with RAPD Method

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각종 작물에서 분리한 *R. solani* 균주들의 RAPD를 이용한 종내 그룹의 유전적 유연관계 분석 및 AGs 신속 간이동정

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ABSTRACT: ***Rhizoctonia solani* [*Thanatephorus cucumeris* (frank) Donk], one of the major soil-borne plant pathogens with world-wide distribution, can cause great damages on various crops. In Korea, sheath blight on rice caused by this pathogen is the major concern, and active studies on this pathogen have been performed. However, most of these studies were concerned with pathogenicity of the isolates instead of molecular analyses of different AGs of *R. solani*. Therefore, in this study, thirty isolates of *Rhizoctonia solani* collected from various sources were used for the analyses of genetic relationships among themselves and for the rapid anastomosis grouping with RAPD method. As a result, thirty isolates of known and unknown AGs were grouped into five subgrups and each group included AG-1, AG-2, AG-3, AG-4, and AG-5. RS-1 isolate was found to be closely realated to AG-5. Isolates RS-4, RS-14, RS-17, and RS-16 were found to be closely realated to AG-2-2(III B). Isolate RS-13 was closely related to AG-4, isolates RS-8 and RS-10 were closely related to AG-1(I B), and isolates RS-7 and RS-21 were closely related to AG-2-2(IV). Isolate RS-19 was closely related to AG-1(I C), and isolates RS-3, RS-5, RS-18, RS-6, and RS-15 were found to be closely related to AG-1.**

KEYWORDS: ***Rhizoctonia solani*, RAPD method**

The genus *Rhizoctonia* was described by Decandolle in 1815 (Sneh *et al.*, 1998). Decandolle designated *R. crocorum* (Pers.) DC. as the type species, while *Rhizoctona solani* was described by Kuhn in 1858 (Sneh *et al.*, 1998). The history of *R. solani* is almost as long as the history of plant pathology. Since these initial reports, the pathology, taxonomy, ecology, and disease control of *Rhizoctonia* have been extensively studied. *R. solani* [*Thanatephorus cucumeris* (frank) Donk], one of the major soil-borne plant pathogens with world-wide distribution and the most important species of *Rhizoctonia*, can cause diseases such as sheath blight, damping-off, brown patch, brown rot, leaf rot, or root rot on different crops.

Cultural characteristics and anastomosis groupings are the main basis for the differention of species within *R. solani*, and more than 20 species of *R. solani* were differentiated

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based on the morpholgical, ecological, enzymatic characteristics and hyphal anastomosis (Carling, 1996, Ogoshi, 1987; Snech *et al.*, 1991a). Hyphal anastomosis reaction provided a major means of differention of *R. solani*, and gave much information on the diversity within the same species. Also, information on the differences in host specificity among the anastomosis groups played a major role in the determination of AGs for the mutant strains used for disease resistant breeding (Matsumoto *et al.*, 1932). However, there are few *R. solani* isolates that do not show anastomosis reaction with known tester strains, and do not belong to any group. In these cases, it takes much time and efforts for anastomosis grouping. Eventhough anastomosis grouping is a useful method for differentiation of *R. solani* isolates, it requires more direct and economic method for the study of biological and pathological aspects of *R. solani* (Kim, 1994, 1996).

Direct analysis of DNA polymorphism is a more general approach to establishing genetic variation in organisms (Kuninaga and Yokozawa, 1980). Moelcular methods involving the use of the PCR (Polymerase Chain Reaction) have been recently described to resolve genetic variation among strains (Choi *et al.*, 1998, Woo *et al.*, 1998). The analysis of random amplified polymorphic DNA (RAPD) was proposed for discrimination between isolates of several fungi including *R. solani*. RAPD amplifies specific DNA segments, and this amplification products can be used for genetic mapping, identification of isolates, and study of genetic variation. In this study, we evaluated the use of RAPD method for genetic analyses of isolates of known and unknown AGs of *R. solani*, and the usefulness of RAPD for rapid discrimination of unknown AGs from known AGs of *R. solani*.

Materials and Methods

Fungal strains

R. solani isolates used in this study were obtained from Agrichemical Screening Division at Korea Research Institute of Chemical Technology, and Agricultural Science and Technology Institute, RDA (Table 1). Collected isolates were cultured on PDA (Potato Dextro Agar) for 5~7 days before they were transferred to PDB (Potato Dextro Broth) for mycelial growth at 25°C for 2 days at 150 rpm (Lee, 1990).

DNA extraction

For large-scale preparations of pure DNA, fungal strains were cultivated at 25°C for 2 days with shaking (150 rpm) in 500 m*l* of potato-dextrose broth inoculated wtih a spore suspension. The mycelium was harvested by filtration, washed, lyophilized, and ground with 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 (500 m*l* Nacl, 100 mM Tris-Hcl pH 8.0, 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 (10 mM Tris-HCl, pH 8.0 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). One unit of Taq polymerase (Dynazyme™) was then mixed and final volume 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, 2 min at 72°C for 45 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 transillumintor.

Table 1. Isolates of *Rhizoctonia solani* used for randomly amplified polymorphic DNA (RAPD) analysis

Isolate No.	*Rhizoctonia solani*	Sources
RS-1	*Rhizoctonia solani*	*Zea mays* L.
RS-3	*Rhizoctonia solani*	*Oryza sativa* L.
RS-4	*Rhizoctonia solani*	*Oryza sativa* L.
RS-5	*Rhizoctonia solani*	*Oryza sativa* L.
RS-6	*Rhizoctonia solani*	*Oryza sativa* L.
RS-7	*Rhizoctonia solani*	*Solanum tuberosum* L.
RS-8	*Rhizoctonia solani*	*Lactuca sativa* L.
RS-10	*Rhizoctonia solani*	*Capsicum annuum* L.
RS-13	*Rhizoctonia solani*	*Brassica oleracea* var. *captitata* L.
RS-14	*Rhizoctonia solani*	*Cucurbita* spp.
RS-15	*Rhizoctonia solani*	unknown
RS-16	*Rhizoctonia solani*	*Monochoria vaginalis* var. *plantaginea*
RS-17	*Rhizoctonia solani*	unknown
RS-21	*Rhizoctonia solani*	unknown
RS-18	*Rhizoctonia solani* AG-1	unknown
CK17	*Rhizoctonia solani* AG-1	unknown
40104	*Rhizoctonia solani* AG-1(IA)	*Cyperus exaltatus* var. *iwasakii* T. Koyama (Sheath blight)
40107	*Rhizoctonia solani* AG-1(IB)	*Codonopsis lanceolata* (S. & Z.) Trautv.
40113	*Rhizoctonia solani* AG-1(IC)	*Brassica campestris* ssp. *pekinensis* (Lour.) Olss.
40120	*Rhizoctonia solani* AG-2-1	unknown
RS-19	*Rhizoctonia solani* AG-2-1	unknown
CK137	*Rhizoctonia solani* AG-2-2	unknown
40126	*Rhizoctonia solani* AG-2-2(IIIB)	unknown
40131	*Rhizoctonia solani* AG-2-2(IV)	*Daucus carota* var. *sativa* DC. (Crown rot)
40137	*Rhizoctonia solani* AG-3	*Solanum tuberoson* L. (Black scurf, Stem canker)
CK19	*Rhizoctonia solani* AG-3	unknown
40139	*Rhizoctonia solani* AG-4	*Raphanus sativus* L. (Damping-off)
CK221	*Rhizoctonia solani* AG-4	unknown
CK25	*Rhizoctonia solani* AG-5	unknown
40145	*Rhizoctonia solani* AG-5	*Allium fistulosum* L. (Damping-off)

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

Primer No.	Base sequences (5' to 3')	No. of bands
OPA-1	CCA GCC CTT C	15(7)[a]
OPA-2	TGC CGA CGT G	10(4)
OPA-3	AGT CAG CCA C	10(6)
OPA-5	AGG GGT CTT G	11(6)
OPA-6	GGT CCC TGA C	10(6)
OPA-17	GAC CGC TTG T	9(6)
OPA-18	AGG TGA CCG T	10(5)
OPA-19	CAA ACG TCG G	10(5)
OPA-20	GTT GCG ATC C	11(5)
Total		96(50)

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

Data analysis

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

that was then used to generate a dendrogram (Romesburg, 1984; Sneath and Sokal, 1973).

Results and Discussion

Genomic DNA was extracted from the mycelium of total thirty isolates of *R. solani* with known and unknown AGs, and the amplified genomic DNA showed bands with different molecular weights. For the PCR reaction and for the comparison of RAPD patterns of genomic DNA of *R. solani*, nine primers were used. Sizes of amplified DNA fragments ranged from 0.2 to 3.2 Kbp. Fifty polymorphic bands were observed from the total of ninety-six bands (Fig. 1, Table 2). Bionomial matrix code (1; presence of band, 0; absence of band) was constructed with ninety-six bands reacted with nine primers. Based on the bionomial matrix code constructed, similarity matrix (Table 3) and dendrogram (Fig. 2) was made with NTSYS-Pc (Rohlf, 1990).

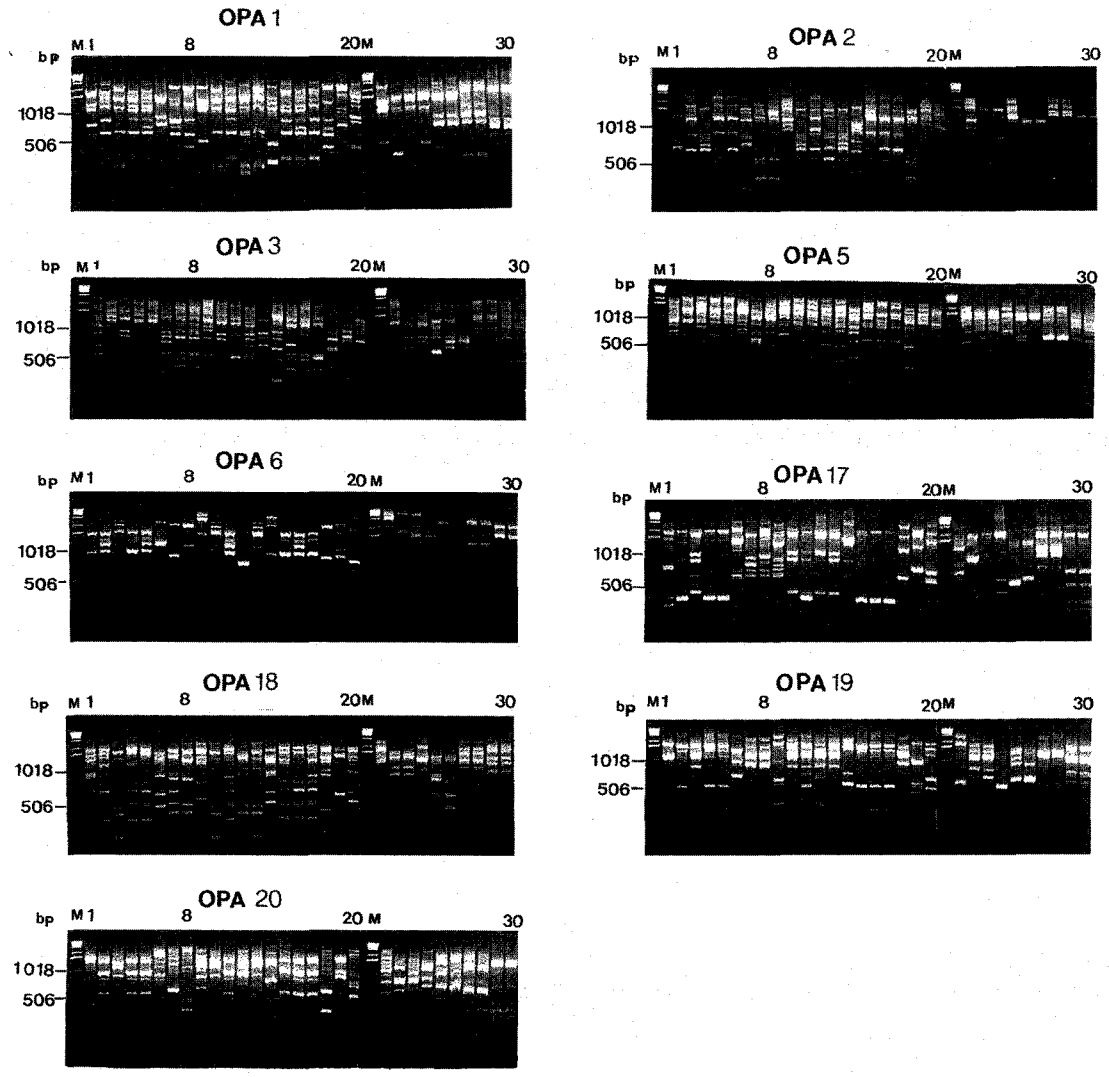

Fig. 1. PCR amplified DNA fragments using the primers listed in Table 2. The numbers on top of the lane indicate the *R. solani* isolates shown in Table 1.

Table 3. Similarity matrix based on the number of shared bands by the compared *Rhizoctonia solani* species with randomly amplified polymorphic DNA[a]

	RS-1	RS-3	RS-4	RS-5	RS-6	RS-7	RS-8	RS-10	RS-13	RS-14	RS-15	RS-16	RS-17	RS-21	RS-18	CK17	40104	40107	40113	40120	RS-19	CK137	40126	401317	CK19	40139	CK221	CK25[b]
RS-3[b]	0.480																											
RS-4	0.540	0.620																										
RS-5	0.580	0.900	0.560																									
RS-6	0.580	0.860	0.520	0.920																								
RS-7	0.580	0.580	0.640	0.480	0.520																							
RS-8	0.560	0.520	0.700	0.540	0.500	0.580																						
RS-10	0.580	0.540	0.720	0.520	0.520	0.600	0.820																					
RS-13	0.640	0.560	0.700	0.580	0.540	0.660	0.640	0.580																				
RS-14	0.540	0.620	0.920	0.600	0.560	0.560	0.620	0.640	0.660																			
RS-15	0.540	0.820	0.560	0.880	0.880	0.440	0.540	0.520	0.500	0.600																		
RS-16	0.500	0.580	0.800	0.600	0.520	0.600	0.660	0.640	0.620	0.760	0.600																	
RS-17	0.560	0.680	0.900	0.620	0.580	0.620	0.640	0.660	0.680	0.860	0.620	0.780																
RS-21	0.580	0.540	0.560	0.560	0.480	0.640	0.660	0.600	0.580	0.480	0.480	0.600	0.540															
RS-18	0.580	0.820	0.560	0.920	0.840	0.440	0.580	0.520	0.580	0.600	0.840	0.560	0.580	0.600														
CK17	0.560	0.840	0.500	0.900	0.980	0.500	0.520	0.500	0.520	0.540	0.900	0.500	0.560	0.500	0.860													
40104	0.560	0.840	0.540	0.860	0.860	0.460	0.560	0.500	0.560	0.540	0.820	0.540	0.560	0.540	0.820	0.880												
40107	0.560	0.480	0.660	0.500	0.500	0.500	0.840	0.780	0.600	0.620	0.500	0.580	0.640	0.620	0.500	0.480	0.520											
40113	0.620	0.500	0.440	0.520	0.520	0.600	0.540	0.400	0.660	0.480	0.560	0.480	0.500	0.600	0.560	0.540	0.580	0.500										
40120	0.520	0.520	0.540	0.500	0.460	0.580	0.560	0.540	0.640	0.500	0.500	0.620	0.520	0.660	0.460	0.440	0.560	0.560	0.620									
RS-19	0.600	0.520	0.460	0.580	0.540	0.460	0.440	0.500	0.720	0.540	0.500	0.500	0.520	0.580	0.540	0.520	0.600	0.600	0.660	0.640								
CK137	0.580	0.540	0.720	0.560	0.520	0.640	0.740	0.680	0.780	0.720	0.520	0.720	0.780	0.560	0.520	0.500	0.540	0.700	0.640	0.620	0.660							
40126	0.520	0.600	0.780	0.540	0.540	0.620	0.680	0.660	0.720	0.780	0.540	0.700	0.840	0.540	0.500	0.520	0.560	0.720	0.540	0.600	0.640	0.900						
40131	0.540	0.540	0.640	0.480	0.440	0.640	0.540	0.520	0.700	0.640	0.400	0.640	0.580	0.720	0.480	0.420	0.540	0.580	0.520	0.700	0.660	0.600	0.660					
40137	0.620	0.620	0.640	0.640	0.600	0.640	0.700	0.680	0.580	0.600	0.560	0.680	0.660	0.680	0.600	0.580	0.580	0.660	0.520	0.700	0.500	0.680	0.660	0.600				
CK19	0.600	0.560	0.660	0.580	0.540	0.700	0.720	0.700	0.640	0.580	0.460	0.620	0.640	0.740	0.620	0.520	0.480	0.640	0.540	0.560	0.440	0.620	0.560	0.580	0.860			
40139	0.580	0.540	0.600	0.520	0.560	0.640	0.500	0.440	0.740	0.600	0.480	0.560	0.700	0.520	0.520	0.540	0.500	0.500	0.640	0.580	0.620	0.680	0.700	0.560	0.560	0.580		
CK221	0.620	0.500	0.640	0.480	0.520	0.600	0.580	0.520	0.780	0.640	0.400	0.520	0.700	0.480	0.480	0.500	0.500	0.540	0.640	0.540	0.620	0.760	0.740	0.560	0.600	0.620	0.920	
CK25	0.680	0.400	0.580	0.420	0.460	0.540	0.600	0.620	0.640	0.580	0.420	0.540	0.560	0.380	0.420	0.440	0.440	0.640	0.500	0.560	0.520	0.660	0.680	0.540	0.500	0.480	0.540	0.580
40145	0.660	0.420	0.640	0.440	0.480	0.520	0.620	0.640	0.620	0.600	0.440	0.560	0.620	0.400	0.440	0.460	0.460	0.660	0.480	0.580	0.500	0.680	0.700	0.520	0.560	0.500	0.560	0.600	0.940

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

[b]Isolate numbers.

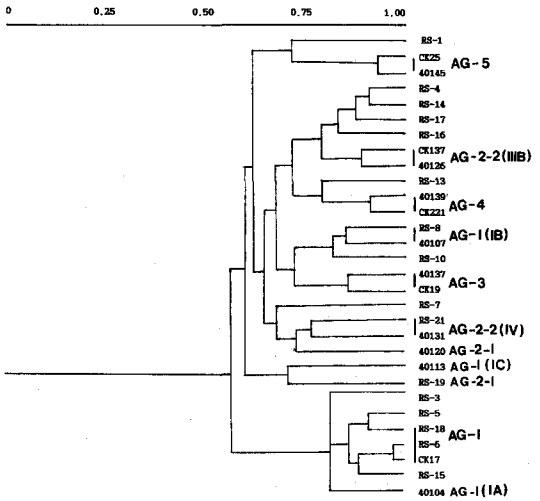

Fig. 2. UPGMA dendgogram showing the relationship among the thirty *Rhizoctonia solani* isolates based on the polymorphic bands formed in 1.5% ararose gel in RAPD analysis.

As shown in dendrogram in Fig. 2, *R. solani* isolates were differentiated into five large groups, and the large five groups were divided into subgroups which were identical with AG group. Isolates RS-18 and CK17 showed similarity of 0.86 within AG-1, and isolates RS-3, RS-5, RS-6, RS-15, and 40104 included in this AG-1 group. Isolates RS-18 and RS-5, CK17 and RS-6, RS-15 and CK17, and RS-3 and RS-5 showed high similarities of 0.920, 0.980, 0.900, and 0.900, respectively. Isolates 40104 which belongs to AG-1(I A) showed similarity of 0.880 with CK17. Isolate 40107 which belongs to AG-1(I B) showed similarities of 0.840 and 0.820 with isolates of RS-8 and RS-10, respectively. AG-3 isolates CK19 and 40137 showed similarity of 0.860, and these isolates showed similarity of 0.700 with AG-1 (I B) group. AG-2-2(IV) isolate 40131 showed similarities of 0.720 and 0.700 with isolates of RS-21 and AG-2-1 isolate 40120, respectivey. AG-2-2(I B) isoaltes CK137 and 40126 showed high similarity of 0.900 between each other, and these isolates showed similarities of 0.840, 0.780, and 0.700 with RS-17, RS-14 and RS-4, and RS-16, respectively. AG-4 isolate 40139 and CK221 showed similarity of 0.920 between each other, and these isolates showed similarity of 0.780 with RS-13. AG-5 isolates CK25 and 40145 showed similarity of 0.940 between each other, and these isolates showed similarity of 0.680 with RS-1 isolate. AG-1(I B) isolate 401707 showed low similarities of 0.500 and 0.480 with AG-1 isolates RS-18 and CK17, respectively. A-2-2(III B) isolate 40126 and AG-2-2(IV) isolate 40131 showed low similarity of 0.660 between each other.

Thirty *R. solani* isolates used in this study were collected from various locations and sources and their AGs were known or unknown. In Korea, *R. solani* isolates obtained from sixty-eight crop species were grouped into AG-1, AG-2-1, AG-2-2, AG-3, AG-4 and AG-5. Furthermore, AG-1 subdivided into I A, I B and I C, and AG-2-2 subdivided into III B and IV. In this study, the results showed I A, I B and I C did not included in AG-1 group. Instead, AG-1(I B) was closely related to AG-3, and AG-1(I C) was closely related to AG-2-1. Also, AG-2-2(III B) and AG-2-2(IV) were found to be closely related to other group. Among the isolates with the same host *Oryza sativa* L., RS-3, RS-5 and RS-6 were included in AG-1 group, and only RS-4 isolate was included in AG-2-2(III B). Comparison of morphological characteristics, presence or absence of anastomosis reaction, and differences in host specificity can be the major means of classification or differentiation of *R. solani* isolates. However, in this study, RAPD method was found to be useful for rapid classification of *R. solani* isolates. Twelve unknown AGs of *R. solani* were rapidly grouped into each AGs based on the PCR reaction results. Other molecular methods such as RFLP, PCR-RFLP, or AFLP analysis would be necessary for further thorough supplement of RAPD method used in this study, or for the development of molecular markers for each different AG for rapid and accurate groupings.

적 요

*Rhizoctonia solani*의 종내 그룹의 분류에는 균사융합군과 배양형태가 많이 이용되고 있으며, 이미 20종 이상의 *R. solani*가 생태학적, 형태학적, 효소학적, hyphal anastomosis 등에 의해 이미 구분되어졌다. Anastomosis group은 *R. solani*를 분리하는데 유용하지만 *R. solani*의 생물학적과 병리학적 연구를 위한 유전적 특성과 동정의 직접적인 방법이 요구되어진다. RAPD는 특별한 DNA 절편을 증폭하고 이를 genetic mapping, identification of isolates에 유용하게 적용될 수 있으며, 또한 genetic variation 조사에도 사용될 수 있다. Dendrogram을 작성한 결과 크게 5 group으로 나뉘어졌고, 5개의 group은 AG group의 subgroup과 동일하게 나뉘어졌다. AG group에 구분되지 않은 species들도 RAPD결과 AG tester들과 grouping되어졌다. RS-1은 AG-5 group에 속하며, RS-4, RS-14, RS-17, RS-16은 AG-2-2(III B)에 속하였다. RS-13은 AG-4에 속하였으며, RS-8과 RS-10은 AG-1(I B)에, RS-7과 RS-21은 AG-2-2(IV) 에 속하였다. RS-19는 AG-1-1(I C)에 속하고, RS-3, RS-5, RS-18, RS-6, RS-15는 AG-1에 속하였다.

RAPD 결과 AG group의 subgroup간의 차이를 볼 수 있었고, 이에 AG group 되어 있지 않은 species의 AG grouping이 이루어졌다. 또한 subgroup 간의 유전적 차이를 확인 할 수 있는 marker를 개발하거나 subgroup의 특별한 primer를 제작하는 SCAs 기법을 이용하여 식물체 병반 또는 토양에서 분리된 *R. solani*를 간이 동정에 이용할 수 있을 것으로 기대한다.

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