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Note

Taxonomic Position of Korean Isolates of *Rhizoctonia solani* Based on RAPD and ITS Sequencing of Ribosomal DNA

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Taxonomic position of 46 Korean isolates of *Rhizoctonia solani* which were classified into nine intraspecific groups by anastomosis and cultural characteristics was analyzed by randomly amplified polymorphic DNA (RAPD) and sequence analyses of the internal transcribed spacer (ITS) regions of ribosomal DNA. All the isolates within each group showed highly similar band patterns in RAPD. The ITS regions of the isolates within the same groups showed a high level of sequence similarity above 96.0% whereas similarities among different groups were below 94.4%. When compared with several reference strains of *R. solani* from foreign countries, all the Korean isolates were clustered with the foreign isolates belonging to the same groups in the phylogenetic tree. All six Korean strains of AG-4 were identified as HG-1 out of 3 subgroup of AG-4. We discussed taxonomic position of Korean isolates of *R. solani* and showed that sequence analysis with ITS regions could be a rapid and useful method for identification of intraspecific group of *R. solani*.

Keywords **:** anastomosis, ITS, randomly amplified polymorphic DNA, *Rhizoctonia solani*

Rhizoctonia solani Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk], the most recognized species within genus *Rhizoctonia*, is potent pathogen of economically important plant species and shows considerable diversity in morphology, geographic location, host specificity and pathogenicity (Ogoshi, 1987). Kim et al. (1994; 1995) reported that *R. solani* had caused 65 diseases to wide range of plants including rice, potato, pepper, vegetables, ornamentals, turf grasses, pine tree etc. in Korea. The concept of anastomosis group (AG) is a widely accepted principle for identifying intraspecific groups in the *R. solani* complex (Carling, 1996). Fourteen AGs, AG-1 through AG-13 and AG-BI (bridging isolates), had been described for *R. solani* by this concept (Carling, 1996; Ogoshi, 1987). However, AG concept is not an ideal method for classification of *R. solani* as misidentification is caused from the varied frequency of hyphal fusion in some AG (Liu and Sinclair, 1992).

For the biological bases of AG, vitamin requirement analysis (Ogoshi and Ui, 1979), serological studies (Adams and Butler, 1979), fatty acid analyses (Stevens Johnk and Jones, 1993; Stevens Johnk et al., 1993), isozyme analyses (Reynolds et al., 1983), total soluble protein pattern (Liu and Ge, 1988), GC content analysis and DNA-DNA hybridization (Kuninaga and Yokozawa, 1980) had been studied. Recently, molecular biological techniques have been used in combination with morphological and physiological markers for the analysis of *R. solani* population (Guleria et al., 2007). The restriction fragment length polymorphism (RFLP) studies on ribosomal DNA regions were performed to differentiate AGs and their subgroups (O'Brien, 1994; Vilgalys and Gonzales, 1990). Randomly amplified polymorphic DNA PCR (RAPD-PCR) analysis was performed to detect genetic variability among the isolates of *R. solani* (Sharma et al., 2005). Sequence analyses of the internal transcribed spacer (ITS) regions of ribosomal DNA have been used to study the genetic relationships between AGs of *R. solani* by many authors (Boysen et al., 1996; Gonzales et al., 2001; Kuninaga et al., 1997; 2000; Salazar et al., 1999).

Several studies were performed with Korean isolates of the species. Kim et al. (1994; 1995) reported cultural characteristics of *R. solani* isolates collected from various crop plants in Korea and Hong et al. (1998) performed PCR-RFLP analysis of their partial 18S-ITS-5.8S region of ribosomal DNA. However, there has been no report on the taxonomic position of Korean isolates using sequence analysis of ribosomal ITS region. In this study, we examined genetic diversity of 46 Korean isolates of *R. solani*, representing 9 groups (6 anastomosis group and 5 cultural types) using randomly amplified polymorphic DNA (RAPD) and DNA sequences of ribosomal ITS region. The objectives of this study were to: (1) determine the complete DNA sequence of the ribosomal ITS regions of Korean isolates, (2) confirm the relatedness between molecular

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evidences and previous identification by anastomosis and cultural characteristics, and (3) examine the taxonomic position of Korean isolates within *R. solani* complex.

Forty-six *Rhizoctonia solani* isolates, representing 6 anastomosis groups (AGs) and 5 cultural types (CTs), were used in this study (Table 1). For RAPD analysis, PELF(5'-ATA TCA TCG AAG CCG C-3') and URP1F (5'-ATC CAA GGT CCG AGA CAA CC-3') by Kang et al. (2002) were used. Ribosomal ITS region was amplified with ITS1 (5'-TTC GTA GGT GAA CCT GCG G-3')

Table 1. The isolates of *Rhizoctonia solani* used in this study

Isolate no.	AG, subgroup	Host	Origin	Sequence accession no.*	Reference
KACC 40101	AG-1, IA	*Oryza sativa*	Korea	KA004124	This study
KACC 40102	AG-1, IA	*Zea mays*	Korea	KA004125	This study
KACC 40103	AG-1, IA	*Arachis hypogaea*	Korea	KA004126	This study
KACC 40104	AG-1, IA	*Cyperus exaltatus* var. *iwasakii*	Korea	KA004127	This study
KACC 40106	AG-1, IA	*Oryza sativa*	Korea	KA004129	This study
A-10	AG-1, IA	*Oryza sativa*	Japan	AB000010	Kuninaga et al. (1997)
KACC 40107	AG-1, IB	*Codonopsis lanceolata*	Korea	KA004130	This study
KACC 40108	AG-1, IB	*Lactuca sativa*	Korea	KA004131	This study
KACC 40109	AG-1, IB	*Cucurbita moschata*	Korea	KA004132	This study
KACC 40110	AG-1, IB	*Nicotiana tabacum*	Korea	KA004133	This study
KACC 40111	AG-1, IB	*Cucumis sativus*	Korea	KA004134	This study
KACC 40112	AG-1, IB	*Lactuca sativa*	Korea	KA004135	This study
001-7	AG-1, IB	Soil	Japan	AB000025	Kuninaga et al. (1997)
KACC 40113	AG-1, IC	*Brassica campestris* ssp. *pekinensis*	Korea	KA004136	This study
KACC 40115	AG-1, IC	*Chaenomeles sinensis*	Korea	KA004137	This study
KACC 40116	AG-1, IC	Soil	Korea	KA004138	This study
KACC 40117	AG-1, IC	*Brassica oleracea* var. *capitata*	Korea	KA004139	This study
KACC 40118	AG-1, IC	Soil	Korea	KA004140	This study
PS-1	AG-1, IC	*Beta vulgaris*	Japan	AB000029	Kuninaga et al. (1997)
3Rs	AG-1, IC	*Pinus densiflora*	Canada	AF354058	Gonzalea et al. (2001)
KACC 40119	AG-2-1	*Brassica campestris* ssp. *pekinensis*	Korea	KA004162	This study
KACC 40120	AG-2-1	*Lactuca sativa*	Korea	KA005649	This study
KACC 40121	AG-2-1	*Tulipa gesneriana*	Korea	KA005650	This study
KACC 40122	AG-2-1	*Malva verticillata*	Korea	KA004141	This study
KACC 40123	AG-2-1	*Panax ginseng*	Korea	KA004163	This study
KACC 40124	AG-2-1	*Raphanus sativus*	Korea	KA005651	This study
R123	AG-2-1	*Brassica oleracea* var. *capitata*	Japan	AB000030	Kuninaga et al. (1997)
8Rs	AG-2-1	Soil	Australia	AF354063	Gonzalea et al. (2001)
KACC 40125	AG-2-2, IIIB	*Platycodon grandiflorum*	Korea	KA004142	This study
KACC 40126	AG-2-2, IIIB	*Angelica gigas*	Korea	KA005652	This study
KACC 40127	AG-2-2, IIIB	*Citrullus lanatus*	Korea	KA005653	This study
KACC 40128	AG-2-2, IIIB	*Citrullus lanatus*	Korea	KA004164	This study
KACC 40129	AG-2-2, IIIB	*Cyperus exaltatus* var. *iwasakii*	Korea	KA005654	This study
KACC 40130	AG-2-2, IIIB	*Zingiber officinale*	Korea	KA005655	This study
C-96	AG-2-2, IIIB	*Juncus effusus*	Japan	AB054854	Carling et al. (2002)
KACC 40131	AG-2-2, IV	*Daucus carota* var. *sativa*	Korea	KA004165	This study
KACC 40133	AG-2-2, IV	*Gypsophila elegans*	Korea	KA004143	This study
KACC 40134	AG-2-2, IV	*Angelica gigas*	Korea	KA005656	This study
KACC 40135	AG-2-2, IV	*Daucus carota* var. *sativa*	Korea	KA005657	This study
BC-10	AG-2-2, IV	*Beta vulgaris*	Japan	AB000014	Kuninaga et al. (1997)
RI-64	AG-2-2, IV	*Beta vulgaris*	Brazil	AY270014	

Table 1. Continued

Isolate no.	AG, subgroup	Host	Origin	Sequence accession no.*	Reference
48R	AG-2-2, LP	*Zoysia tennuifolia*	Japan	AB054866	Carling et al. (2002)
RGR38	AG-2-2, LP	*Cynodon dactylon*	Japan	AB054869	Carling et al. (2002)
H5-307	AG-2-3	*Glycine max*	Japan	AB054870	Carling et al. (2002)
LB17-3-2	AG-2-3	*Glycine max*	Japan	AB054872	Carling et al. (2002)
758C	AG-2-4	*Dauscus carota*	USA	AB054879	Carling et al. (2002)
KACC 40136	AG-3	*Solanum tuberosum*	Korea	KA004166	This study
KACC 40137	AG-3	*Solanum tuberosum*	Korea	KA004144	This study
KACC 40138	AG-3	*Solanum tuberosum*	Korea	KA004145	This study
1600	AG-3	*Nicotiana tabacum*	USA	AB000004	Kuninaga et al. (1997)
1600NC	AG-3	*Nicotiana tabacum*	USA	AF153774	Pope & Carter (2001)
FL3	AG-3	*Nicotiana tabacum*	USA	AF153773	Pope & Carter (2001)
OKA-6	AG-3	*Lycopersicon esculentum*	Japan	AB000023	Kuninaga et al. (1997)
ST3-1	AG-3	*Solanum tuberosum*	Japan	AB000041	Kuninaga et al. (1997)
CP245	AG-3	*Solanum tuberosum*	USA	AF153771	Pope & Carter (2001)
K213C	AG-3	*Solanum tuberosum*	Russia	AF153772	Pope & Carter (2001)
T31	AG-3	*Solanum tuberosum*	Spain	AY387528	
CBS 211.84	AG-3	*Solanum tuberosum*	Netherlands	AY387574	
KACC 40139	AG-4, HG-I	*Raphanus sativus*	Korea	KA004146	This study
KACC 40140	AG-4, HG-I	*Cucumis sativus*	Korea	KA005658	This study
KACC 40141	AG-4, HG-I	*Capsicum annuum*	Korea	KA005659	This study
KACC 40142	AG-4, HG-I	*Cucumis melo*	Korea	KA004147	This study
KACC 40143	AG-4, HG-I	*Dianthus caryophillus*	Korea	KA004148	This study
KACC 40144	AG-4, HG-I	*Spinacia oleracea*	Korea	KA004149	This study
78-23R-3	AG-4, HG-I	*Spinacia oleracea*	Japan	AB000007	Kuninaga et al. (1997)
R97	AG-4, HG-I	*Beta vulgaris*	Japan	AB000031	Kuninaga et al. (1997)
Chr-3	AG-4, HG-I	*Chrysanthemum morifolium*	Japan	AB000015	Kuninaga et al. (1997)
Rh-131	AG-4, HG-II	*Beta vulgaris*	Japan	AB000031	Kuninaga et al. (1997)
7Rs	AG-4, HG-II	*Medicago sativa*	USA	AF354074	Gonzalea et al. (2001)
18Rs	AG-4, HG-II	*Beta vulgaris*	Japan	AF354072	Gonzalea et al. (2001)
6Rs	AG-4, HG-III	Conifer	USA	AF354077	Gonzalea et al. (2001)
45Rs	AG-4, HG-III	*Beta vulgaris*	USA	AF354076	Gonzalea et al. (2001)
KACC 40145	AG-5	*Allium fistulosum*	Korea	KA004150	This study
KACC 40146	AG-5	*Panax ginseng*	Korea	KA004151	This study
KACC 40147	AG-5	*Iris nerpschinskia*	Korea	KA004152	This study
KACC 40148	AG-5	*Zingiber officinale*	Korea	KA005660	This study
KACC 40150	AG-5	*Brassica oleracea* var. *capitata*	Korea	KA005661	This study
K31	AG-5	Pine tree	Japan	AB000021	Kuninaga et al. (1997)

*The sequence accession numbers, KAxxxxxx, of ribosomal ITS regions were from Korean Agricultural Culture Collection (KACC, http://kacc.rda.go.kr), and the other numbers from GenBank of NCBI.

and ITS4 (5'-AAC ATG CGT GAG ATT GTA AGT-3') primers (White et al., 1990) and sequenced in Solgent co. Ltd. The ITS sequences aligned by Clustal W (Thompson et al., 1994) and, phylogenetic tree was constructed using MEGA version 4 (Tamura et al., 2007) with reference sequences from GenBank and sequence of *Rhizoctonia alpina* (CBS 309.35) was used as an outgroup. Distances were calculated using the Tamura-Nei parameter model and a phylogenetic tree was constructed through Neighbor-Joining (NJ) analysis. Bootstrap analysis was performed with 1,000 re-samples of data.

Interrelation between intraspecific groups and molecular characteristics. Forty-six *Rhizoctonia solani* isolates

were analyzed by RAPD using two primers, PELF and URP1F and both primers produced multiple polymorphic bands. Although there were some differences in minor bands, isolates in the same AG showed the identical band patterns (Fig. 1). The length of ribosomal ITS regions varied as AGs due to various length of ITS1 (198-237 bp) and ITS2 (269-289 bp). Except for two subgroups of AG-2-2, sequence similarities of the Korean isolates were above 98.7% in the same groups but below 94.4% among the different groups. Phylogenetic analysis using ITS region with 730 bp length including gaps showed that isolates belonging to the different groups formed completely distinct clusters. When compared with 32 reference isolates from foreign countries listed in Table 1, all the Korean isolates were well clustered with them by AGs (Fig. 2). Isolates of three subgroups of AG-1 clustered with reference isolates of each subgroups. Four subgroups of AG-2 were phylogenetically distant each other and AG-2-1 and AG-2-4 made a cluster with AG-3, however, AG-2-2 and AG-2-3 made a cluster with AG-5. All isolates of three subgroups of AG-2-2 were well clustered according to their group although the cluster of AG-2-2(IV) had low bootstrap value. Three Korean isolates of AG-3 from potato were much closer to reference isolates from potatoes than those from tobacco and tomato. All the Korean isolates of AG-4 were closed to HG-I among three subgroups of AG-4. Molecular characteristics examined by RAPD analysis and ITS sequencing were strongly interrelated with intraspecific groups by cultural characteristics and anastomosis.

Taxonomic position of Korean isolates within *R. solani* complex. AG-1 was divided into three subgroups based on pathogenicity and cultural characteristics. They were AG-1(IA) causing sheath blight on rice, AG-1(IB) causing web-blight and AG1(IC) causing damping off in host plants (Sneh et al., 1991). Our result from sequence analysis of ribosomal ITS region and RAPD showed that the three subgroups were completely distinguishable from each other. This result was consistent with previous report showing the different PCR-RFLP band patterns of 17S and ITS regions digested by a restriction enzyme *Cfr*13I (Hong et al., 1998).

AG-2 causing root disease on crucifer (Anderson, 1982) had been divided into five subgroups, AG-2-1, AG-2-2, AG-2-3, AG-2-4 and AG-2(BI), on the basis of hyphal fusion frequency (Carling et al., 2002; Ogoshi, 1987). Isolates of AG-2-1 are autotrophic, and isolates of AG-2-2 and AG-2-3 are auxotrophic for thiamine. AG-2-2 is subdivided again into two cultural types, AG-2-2(IIIB) (rush type) and AG-2-2(IV) (root rot type) on the basis of their pathogenicity on mat rush and sugar beet, respectively (Salazar et al., 1999) , and confirmed by analyses of DNA sequence homology (Liu and Sinclair, 1992). In the present study, we examined 16 Korean isolates of AG-2-1, AG-2-2(IIIB), and AG-2-2(IV). Six strains of AG-2-1 were located near to AG-2-4 and AG-3. However, 6 strains of AG-2-2 (IIIB) and 4 strains of AG-2-2 (IV) were located near to AF-2-3 and AG-2-5 (Fig. 2). It means that AG-2 is not monophyletic. Further physiological and phylogenetical study would be required to understand AG-2. Traditionally, field isolates of AG-3 had been considered to be main pathogens causing stem canker, stolon lesion, and black sclerotia on tubers of potato (Anderson, 1982). However, Date et al. (1984) and Stevens Johnk et al.

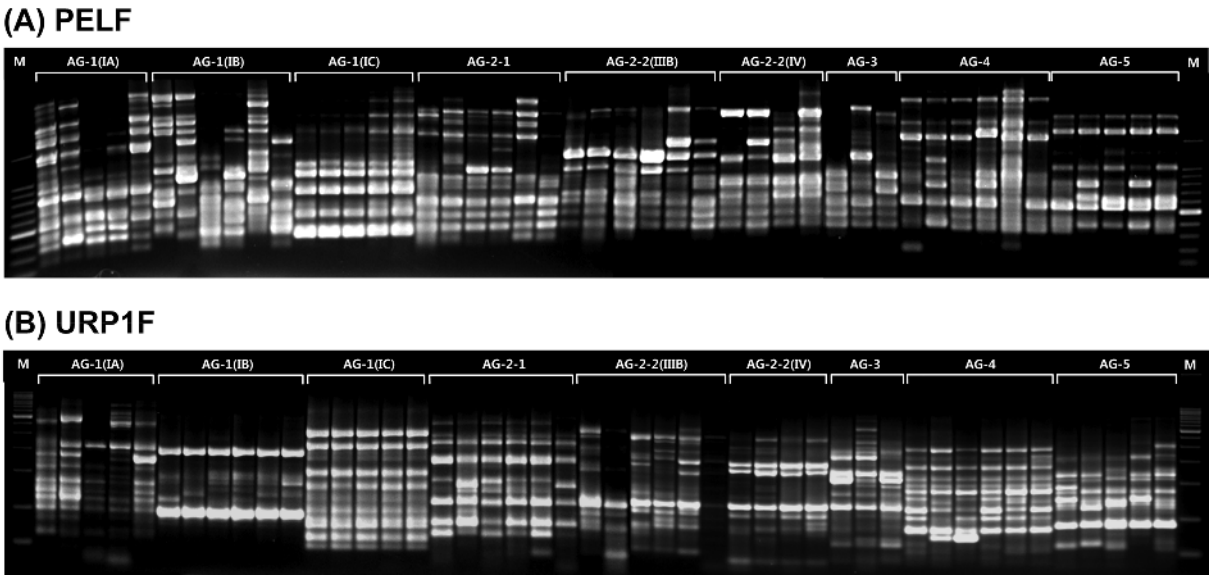

Fig. 1. RAPD band pattern of the Korean isolates of *Rhizoctonia solani* generated by PELF and URP1F primers. The texts above the lanes represent intraspecific groups.

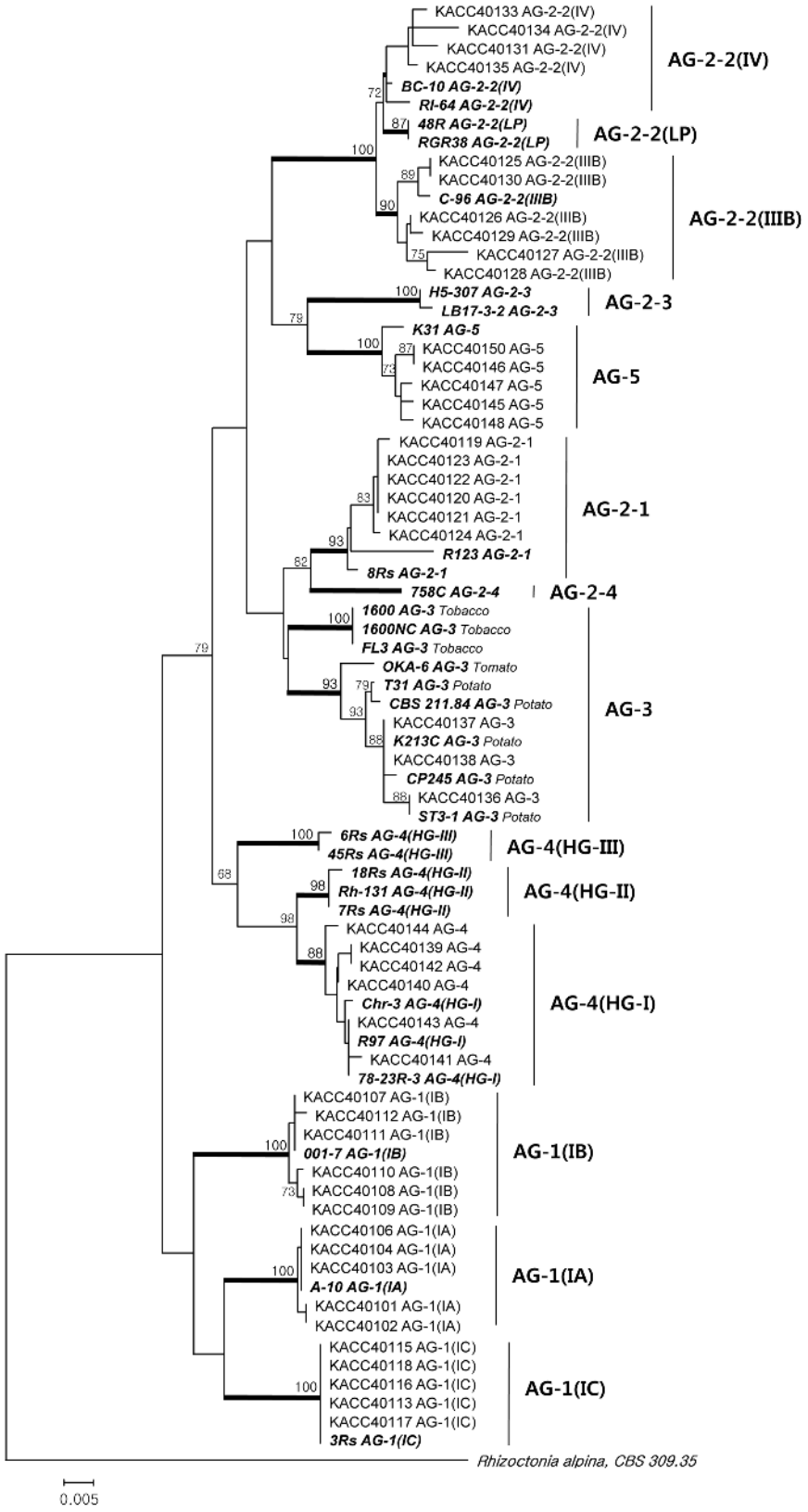

Fig. 2. Neighbor-Joining tree of the Korean isolates of *R. solani* with reference isolates (in bold) based on the sequence of ribosomal ITS region. The numbers above or below the nodes represent bootstrap values of >60% (out of 1,000 bootstrap replication). The number of nucleotide changes is represented by branch length. CBS 309.35 (*R. alpina*) was used as outgroup.

(1993) reported that isolates of AG-3 caused leaf blight of tomato and target spot of tobacco, respectively. Several authors reported previously differences in fatty acid, isozyme composition, sequence variation of ribosomal ITS region and pathogenicity between AG-3 isolates originated from tobacco and potato (Carling and Leiner, 1990; Kuninaga et al., 2000; Stevens Johnk et al., 1993). Three Korean isolates of AG-3 originated from potato were completely clustered with reference isolates from potato, but located distantly from those from tobacco.

Several studies suggested that AG-4 may consist of three homogeneous subgroups, HG-I, HG-II and HG-III, by biochemical and molecular evidences such as fatty acid analysis (Stevens Johnk and Jones, 2001), DNA-DNA reassociation (Kuninaga and Yokozawa, 1984) and nucleotide sequence analysis of ribosomal ITS region (Kuninaga et al., 1997). Based on the sequence of ITS, all Korean isolates of AG-4 were clustered into HG-I. However, any relatedness between subgroups and host plants was not observed. Five isolates of AG-5 were well clustered with reference strain from overseas. This group showed close relationships with AG-2-3.

According to Ogoshi (1987), *R. solani* shows considerable diversity in geographic location and host specificity. However, we did not observe significant relatedness among the sequences of ribosomal ITS region and isolated location in this study. Almost Korean and overseas isolates in the same groups showed high sequence similarity in ribosomal ITS regions. Only AG-3 isolates showed a trend of host specificity in phylogenetic tree with ITS sequences.

For identification of AG and cultural type in *R. solani*, we have usually performed anastomosis test with reference strains from every recognized group and examined their cultural and morphological characteristics. However, it is not easy work because we have to collect reference strains from every group and anstomosis test takes long time and the results are often ambiguous to determine. According to results from this work, ribosomal ITS region was strongly discriminative to AGs of *R. solani* complex. Almost Korean isolates of *R. solani* were classified into nine intraspecific groups by anastomosis and cultural characteristics, and they were well clustered to their reference isolates from overseas in phylogenetic tree with ITS sequences. It means that sequence analysis with ribosomal ITS regions could be a good and rapid method to identify anastomosis groups and cultural types in *R. solani* complex.

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