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FUNGAL DISEASES

Short communication

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Comparison of sequences for the internal transcribed spacer region in *Rhizoctonia solani* AG 1-ID and other subgroups of AG 1

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Abstract The rDNA-ITS sequence of *Rhizoctonia solani* AG 1-ID was determined and compared to those of *R. solani* AG 1-IA, AG 1-IB, and AG 1-IC. The similarity of the isolates from each AG 1 subgroup was almost identical (99%–100%), whereas it was lower between subgroups (91%–95%) than within subgroups. Phylogenetic analysis indicated that isolates of AG 1-ID and other subgroups were separately clustered. Isolates of *R. solani* AG 1 were clearly separated from *R. solani* AG 2-1, AG 4, and binucleate *Rhizoctonia* AG-Bb and AG-K. These results showed that analysis of the rDNA-ITS sequence is an optimal criterion for differentiating *R. solani* AG 1-ID from other subgroups of *R. solani* AG 1.

Key words *Rhizoctonia solani* AG 1-ID · rDNA-ITS sequence · Phylogenetic analysis

Rhizoctonia solani Künh [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is an important soilborne fungal pathogen for a wide range of host plants. *R. solani* consists of 14 anastomosis groups (AG 1–13) based on hyphal anastomosis behavior. Based on cultural morphology, pathogenicity, and molecular analyses, anastomosis group 1 (AG 1) of *R. solani* has been further divided into three subgroups, AG 1-IA (rice sheath blight), AG 1-IB (web blight), and AG 1-IC (damping off) (Hyakumachi and Sumino 1984; Jones and Belmar 1989; Sneh et al. 1991). The causal agent of a necrotic leaf spot on coffee in the Philippines was identified as a new subgroup of *R. solani* AG 1, namely AG 1-ID (Priyatomojo et al. 2001). Isolates of AG 1-ID could be distinguished from other subgroups based on cultural characteristics, fatty acid analysis, random amplified polymorphic DNA (RAPD) analysis, and restriction fragment length polymorphism (RFLP) analysis of the internal transcribed spacer region of ribosomal DNA (rDNA-ITS region) (Priyatomojo et al. 2001). As an identification method, however, analyses of fatty acids and RFLP of rDNA-ITS could not clearly distinguish some subgroups within AG 1 (Priyatomojo et al. 2001). For RAPD analysis, polymerase chain reaction (PCR) products amplified by RAPD-PCR are known to have a problem with reproducibility. Among molecular tools, the sequence of the rDNA-ITS region was used for establishing the phylogenetic relations among similar cultural types within AG 2-2 (Salazar et al. 2000). We therefore analyzed the complete sequence of the rDNA-ITS regions of *R. solani* AG 1-ID to compare it with three other subgroups of AG 1.

Nineteen isolates of *R. solani* AG 1 were used to determine the complete sequence of the rDNA-ITS region. Two other isolates of *R. solani* (AG 2-1 and AG 4) and two isolates of binucleate *Rhizoctonia* (AG-Bb and AG-K) were used for comparison. DNA of each isolate was extracted using the method of Priyatomojo et al. (2001). PCR with primers ITS 1 (5-TCCGTAGGTGAACCTGCGG-3) and ITS 4 (5-TCCTCCGCTTATTGATATGC-3) described by White et al. (1990) was used to amplify rDNA-ITS regions of all used isolates. The PCR products of the rDNA-ITS region were sequenced using primers ITS 1, ITS 2 (5-GCTGCGTTCTTCATCGATGC-3), ITS 3 (5-GCA TCGATGAAGAACGCAGC-3), and ITS4 with a Ready Reaction Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed with an ABI 3100 DNA sequencer (Applied Biosystems). The sequence has been deposited in the GenBank database (accession nos. AB122124, AB122125, AB122126, AB122127, AB122128, AB122129, AB122130, AB122131, AB122132, AB122133, AB122134, AB122135, AB122136, AB122137, AB122138,

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Table 1. Percentage of sequence similarity among *Rhizoctonia solani* AG 1 subgroups, AG 2-1, and AG 4 and binucleate *Rhizoctonia* isolates AG-Bb and AG-K

Isolate	1-ID	1-IA	1-IB	1-IC	2-1	4	Bb	K	*F.s.*
R. solani AG 1									
AG 1-ID	99–100	91	94–95	92	89	89	81	88–89	22
AG 1-IA		99–100	91	92	87–89	88–89	81–83	85–88	22
AG 1-IB			99–100	91–92	87–88	90–91	82	87–88	22
AG 1-IC				99–100	89–90	91	85–86	80–86	22
AG 2-1					100	89	84	86	22
AG 4						100	82	87	22
Binucleate *Rhizoctonia*									
AG-Bb							100	88	22
AG-K								100	22
F.s.									100

F.s., *Fusarium solani* f. sp. *pisi*
Rounded figures for all values from clustalx version 1.8 were used in the table

AB122139, AB122140, AB122141, AB122142, AB122143, AB122144, AB122145).

The rDNA-ITS sequence of *R. solani* AG 1-ID was aligned with those of AG 1-IA, AG 1-IB, AG 1-IC, *R. solani* AG 2-1, AG 4, and binucleate *Rhizoctonia* isolates AG-Bb and AG-K. Sequence similarities were evaluated using clustalx version 1.8 (Thompson et al. 1997). The sequence data for the ITS region from two isolates of AG 1-IA, one isolate of AG 1-IC, and *F. solani* f. sp. *pisi* registered in the GenBank database (accession nos. AF354058, AY154300, AY154301, AF150458) were included for these analyses.

With a direct pairwise comparison of rDNA-ITS sequences, similarity within isolates of AG 1-ID was almost 100% (99%–100%), and those within isolates of each subgroup were also almost identical (AG 1-IA, 99%–100%; AG1-IB, 99%–100%; AG 1-IC, 99%–100%) (Table 1). Although sequence similarities between subgroups of AG1 were lower than within subgroups, similarities were still high (91%–95%). Sequence similarities of AG 1-ID and other AG 1 subgroups with other AGs were moderately high (89% with AG 2-1 and AG 4, 81% with AG-Bb (binucleate), and 88%–89% with AG-K (binucleate)) (Table 1). Sequence similarities of all isolates of *R. solani* and binucleate *Rhizoctonia* with *F. solani* f. sp. *pisi* were 22% (Table 1).

For phylogenetic analysis, a single neighbor-joining tree was obtained using rDNA-ITS region sequences of 26 isolates of *R. solani* AG 1, AG 2-1, and AG 4 and of binucleate *Rhizoctonia* AG-Bb and AG-K, with *F. solani* f. sp. *pisi* as the outgroup (Fig. 1). Isolates of AG 1-ID and other AG 1 subgroups were separately clustered in each of the subgroups. Each of the four branches with bootstrap support was found in the four most parsimonious trees (Fig. 1). All subgroups of AG 1 were closer to isolates B-1 (AG 2-1) and Chin1-1S (AG 4) than to isolates C350 and AC-1 (binucleate *Rhizoctonia* AG-Bb and AG-K, respectively) (Fig. 1).

Sequence homologies of the rDNA-ITS region in isolates of *R. solani* AG 1-ID and other AG 1 subgroups were almost 100% (Table 1). This shows that isolates of *R. solani* AG 1 that belong to the same subgroup have the same

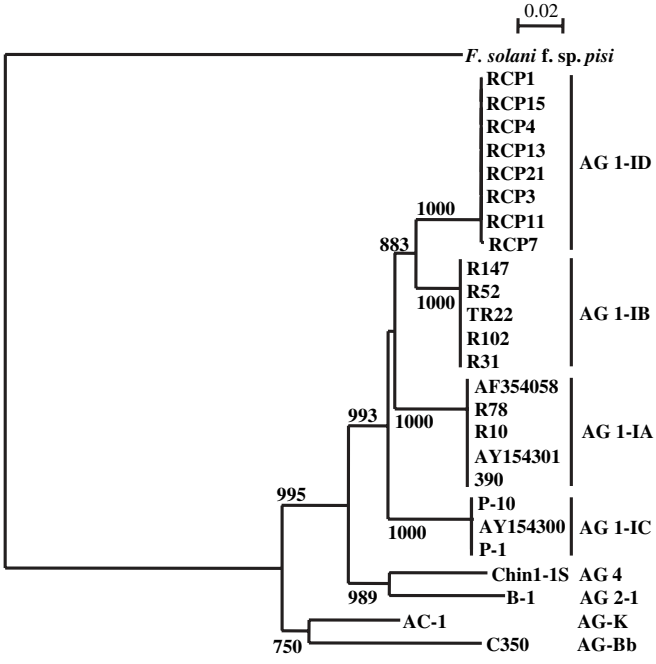

Fig. 1. Phylogenetic distance tree, constructed by the neighbor-joining method, to compare the nearly complete rDNA-ITS region sequence of *Rhizoctonia solani* AG 1-ID with AG 1-IA, AG 1-IB, AG 1-IC, AG 2-1, AG 4, and binucleate *Rhizoctonia* AG-Bb and AG-K. *Fusarium solani* f. sp. *pisi* was used as the outgroup. The numbers on the branches are confidence values obtained for 1000 bootstrap replicates (only values above 70% are shown). AGs and subgroups of each isolate are shown in the right column. *Bar* represents a phylogenetic distance of 2%

sequence in the ITS region. Between subgroups, sequence homologies were still high (91%–95%). Similar results were reported using ITS sequences among three cultural types of *R. solani* AG 2-2 (Salazar et al. 2000). The phylogenetic tree using parsimony based on the ITS sequence showed that each subgroup of *R. solani* AG (1-IA, 1-IB, 1-IC, 1-ID) was distinct by bootstrap support (Fig. 1). A phylogenetic tree was also obtained using RAPD-PCR (Priyatomojo et al. 2001). Although the phylogenetic tree using RAPD-PCR had a shape different from that using the ITS sequence, both trees showed that each AG 1 subgroup was clearly

distinct. By comparing these trees, the phylogenetic distance using the ITS sequence was more accurate among AG 1 subgroups than was that with RAPD-PCR. Our data emphasize that the ITS sequence has enough criteria to differentiate the subgroups among *R. solani* AG 1.

Isolates of *R. solani* AG 2-1 and AG 4 used for comparison had high sequence homologies with isolates of *R. solani* AG 1 (87%–91%). Such high homologies were also observed between AG 1, AG 2-1, AG 4, and binucleate *Rhizoctonia* isolates AG-Bb and AG-K (81%–89%). Similarly, Kuninaga (2002), using neighbor-joining analysis of the ITS sequence, showed that AG 1 (1-IA, 1-IB, 1-IC) and AG 13 clustered together. However, homologies between *Rhizoctonia* (*R. solani* AG 1, AG 2-1, AG 4, and binucleate *Rhizoctonia* isolates AG-Bb and AG-K) and *F. solani* f. sp. *pisi* were significantly low (22%) (Table 1). The sequences of the rDNA-ITS regions appear to be well conserved among *Rhizoctonia* species.

We concluded that analysis of the rDNA-ITS sequence is the most suitable molecular tool for accurately identifying *R. solani* AG 1-ID and other subgroups.

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