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Short communication

Identification of *Rhizoctonia solani* AG 1-IB in lettuce, AG 4 HG-I in tomato and melon, and AG 4 HG-III in broccoli and spinach, in Brazil

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Abstract

Fungi isolated in Brazil, from lettuce, broccoli, spinach, melon and tomato, were identified as *Rhizoctonia solani*. All lettuce isolates anastomosed with both AG 1-IA and IB subgroups and all isolates from broccoli, spinach, melon and tomato anastomosed with AG 4 subgroup HG-I, as well as with subgroups HG-II and HG-III. DNA sequence analyses of ribosomal internal transcribed spacers showed that isolates from lettuce were AG 1-IB, isolates from tomato and melon were AG 4 HG-I, and isolates from broccoli and spinach were AG 4 HG-III. The tomato isolates caused stem rot symptoms, the spinach, broccoli and melon isolates caused hypocotyl and root rot symptoms on the respective host plants and the lettuce isolates caused bottom rot. This is the first report on the occurrence in Brazil of *R. solani* AG 4 HG-I in tomato and melon, of AG 4 HG-III in broccoli and spinach and of AG 1-IB in lettuce.

Rhizoctonia spp. attack agricultural and horticultural crops throughout the world, infecting the seeds, roots, leaves, stems and fruits of many crops. In Brazil, *Rhizoctonia solani* is one of the most important pathogens causing disease in several crops, including vegetables. The number of nuclei per cell of *Rhizoctonia* is an important taxonomic parameter used to divide *Rhizoctonia* species into multinucleate, represented by *R. solani*, and binucleate comprise the other *Rhizoctonia* spp. Fourteen anastomosis groups (AGs), AG 1–AG 14 have been recognized and characterized. Among the 14 AGs, AG 1 has been divided into AG 1-IA, IB, IC and AG 4 into AG 4 HG-I, HG-II, HG-III subgroups. The separation of subgroups AG 1 and AG 4 is not possible by hyphal anastomosis reactions because anastomosis occurs within members from the same AG of *R. solani*. To differentiate isolates within AG 1 and AG 4, biochemical and molecular techniques such as fatty acid analysis (Stevens Johnk and Jones, 2001), DNA based sequence homology (Kuninaga and Yokozawa, 1984), restriction fragment length polymorphism of internal transcribed spacer (ITS) (Liu and Sinclair, 1993; Priyatmojo et al., 2001), random amplified polymorphic DNA (Toda et al., 1999; Priyatmojo et al., 2001) and rDNA-ITS nucleotide sequences (Kuninaga et al., 1997) have been used.

In Brazil, there are few reports about the occurrence of *R. solani* causing disease in vegetable crops. Bolkan and Ribeiro (1985) isolated and characterized *R. solani* from artichoke, peppers (sweet, black and bell), carrot, cabbage, chicory, cucumber, eggplant, lettuce, pea, and sugar beet. Although *Rhizoctonia* has been considered a very important pathogen causing high yield losses in vegetable crops in Brazil, there are no reports describing the AGs and subgroups occurring in those crops. The objectives of this study were to identify AGs of *Rhizoctonia* isolates collected from lettuce, tomato, broccoli, spinach and melon in Brazil and to determine their pathogenicity.

Table 1. Brazilian isolates of *R. solani* from lettuce (AL), broccoli (BR), spinach (EP), melon (ME) and tomato (TO) from different locations

Isolate	Origin	Host	Symptom
AL-1, AL-2, Al-3	Botucatu-SP	*Lactuca sativa*	Bottom rot
BR-1, BR-2, BR-3	Pardinho-SP	*Brassica oleracea* var. *capitata*	Hypocotyl and root rot
EP-1, EP-2, EP-3	Mogi das Cruzes-SP	*Spinacia oleracea*	Hypocotyl and root rot
ME-1, ME-2, ME-3	Botucatu-SP	*Cucumis melon*	Hypocotyl and root rot
TO-1, TO-2	Patos de Minas-MG	*Lycopersicum sculentum*	Stem and fruit rot
TO-3, TO-4, TO-5	Paracatu-MG	*Lycopersicum sculentum*	Stem and fruit rot

The isolates from different regions in Brazil were collected from broccoli, melon, spinach, lettuce and tomato (Table 1). The nuclear conditions and AG of the isolates were determined (Ceresini et al., 1996). All these isolates were multinucleate *R. solani,* with an average of 4.7, 9.3, 5.7, 11.0 and 6.5 nuclei/cell, respectively. Tester isolates of *R. solani* AG 1–AG 9, obtained from researchers from different parts of the world, were paired with all isolates collected. Hyphae were considered to be compatible when at least 5 points on each of 4 slides/isolate showed C2 and C3 type-reactions (Carling and Leiner, 1990). All isolates from broccoli, spinach, melon and tomato anastomosed with isolates of subgroup AG 4 HG-I, AG 4 HG-II and AG 4 HG-III testers. All isolates from lettuce anastomosed with AG 1-IA and IB.

DNA was isolated from a total of 39 isolates including 22 tester isolates (Kuramae-Izioka, 1997). The ITS4/ITS5 primer set (White et al., 1990) was used for PCR amplification of nuclear ITS1 and ITS2 regions and the 5.8S rRNA gene. Each PCR product was purified using MicroSpin S-400 HR columns (Amersham Pharmacia) according to instructions of the manufacturer and sequenced using 10 ng PCR product and 1 μM each of the ITS2, ITS3, ITS4 or ITS5 primers following the protocol supplied with the Amersham Premix Terminator (Amersham Pharmacia). Sequencing was performed using a PE Applied Biosystems Model 377 DNA Sequencer. The four sequenced fragments generated from each isolate were assembled using Phred/Phrap (Ewing et al., 1998) and Consed (Gordon et al., 1998) and all consensus bases were of high quality with a Phred value greater than 20. The consensus sequence was trimmed in order to have only the ITS1-5.8S-ITS2 sequences analyzed. GenBank accession numbers are indicated in Figure 1. The sequences were aligned using the computer software package CLUSTAL X (Thompson et al., 1997). The tree showing the phylogenetic relatedness between isolates and AG testers was constructed from distance matrix values by the neighbor-joining method and 1000 bootstrap values. The computational analysis to generate the phylogenetic relationship tree between representatives AGs and the isolates was performed using PAUP* (Phylogenetic Analysis Using Parsimony, version 4.0b5a) with heuristic search with 50 replicates with tree-bisection-reconnection (TBR) as the branch-swapping algorithm. Figure 1 shows the phylogenetic relationships of isolates and AG testers of *R. solani* what was inferred from neighbor-joining and heuristic parsimony analysis of the aligned ITS1, ITS2 regions and 5.8S rRNA gene sequences. The most parsimony tree obtained by PAUP by analysis of the aligned sequences was also the best tree obtained by the neighbor-joining method. The isolates from tomato and melon were more close related to AG 4 HG-I while isolates from broccoli and spinach were more close related to AG 4 HG-III. The similarity between isolates from tomato and melon was higher to AG 4 HG-I (>98.5%) than to AG 4 HG-II (95.7–96.6%) and to AG 4 HG-III (93.0–93.8%). The occurrence of *R. solani* AG 4 HG-I in tomato causing fruit rot has being also reported by Rahimian (1988) in Iran. Damping off in melon caused by AG 4 was identified by Uematsu et al. (1993) and here the subgroup HG-I was identified in the same AG causing hypocotyl and root rot in melon.

The similarity between isolates from broccoli and spinach was higher to AG 4 HG-III tester (>99.4%) than to AG 4 HG-I (93.5% and 93.6%) and to AG 4 HG-II (93.1% and 93.3%). The same AG 4 isolates from spinach have been characterized by Naiki and Kanoh (1978) in Japan, but not the subgroup. Besides AG 4, AG 5 has also been reported to cause damping off in spinach in Japan (Akashi et al., 1986). The isolates collected here from broccoli with hypocotyl and root rot were identified as AG 4, subgroup HG-III. In China, Chen et al. (1990) observed that in crucifers, AG 2-1 was more frequent in spring, while AG 4 occurred in all four seasons causing damping off.

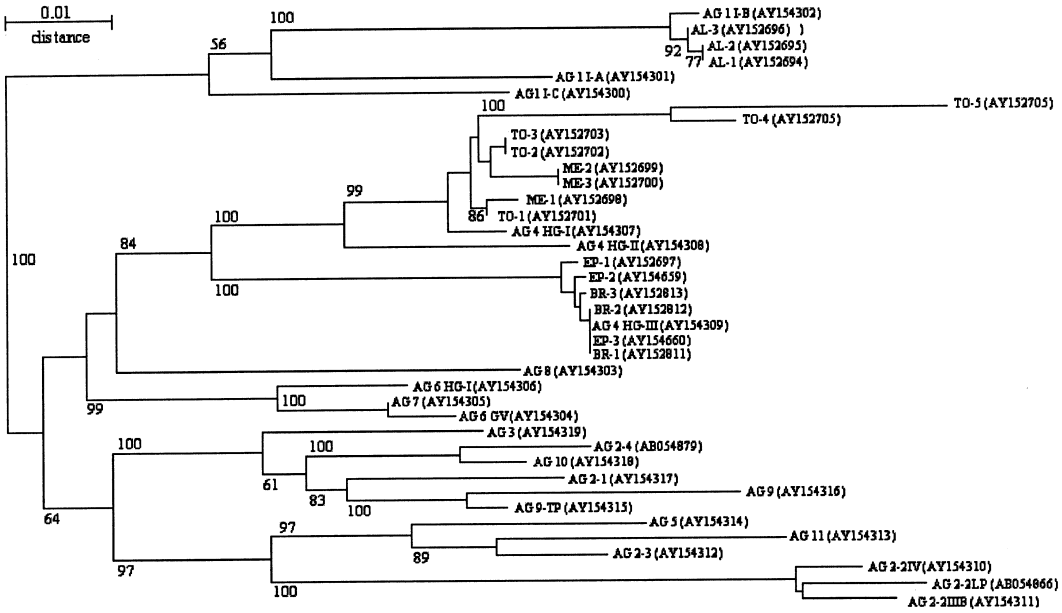

Figure 1. Neighbor-joining tree obtained with 39 aligned ITS1-5.8S-ITS2 sequences illustrating the relationship of isolates of *R. solani* from broccoli (BR), spinach (EP), melon (ME), tomato (TO), lettuce (AL) and AG testers. The numbers at branches indicate the percentage of 1000 bootstrap replications and the numbers between parenthesis are the accession numbers of the isolates submitted to GenBank.

The similarity between isolates from lettuce was higher to AG 1-IB tester (>99.4%) than to AG 1-IA (93.1% and 93.4%) and to AG 1-IC (93.5% and 93.6%). In Japan AG 1-IC has been found to be pathogenic to lettuce seedlings (Hyakumachi and Sumino, 1984) while in the US, AG 1-IB has been found to be more virulent (Herr, 1992). Although *R. solani* has been recognized as a pathogen of lettuce in Brazil (Bolkan and Ribeiro, 1985), there is no report of AG 1 subgroup being responsible for bottom rot symptoms. *Rhizoctonia solani* is one of the most important fungi causing lettuce losses in Brazil (Pinto et al., 1994).

The pathogenicity of the isolates was tested under greenhouse conditions at 25 ± 2 °C. A completely randomized design with five replicate pots per isolate was used. The lettuce isolates leaves were inoculated on cv. Elisa, while the tomato isolates were inoculated on tomato plants cv. Santa Clara according to the method described by Bolkan and Ribeiro (1985). Forty-eight hours after inoculation, the plants were covered with plastic bags. After five days, disease severity was assessed using the scale described by Bolkan and Ribeiro (1985) and the respective fungi were re-isolated. The pathogenicity of the broccoli and melon isolates was determined using a substrate and inoculum preparation (Fenille and Souza, 1999). Twelve seeds of spinach cv. Tohkai, 12 seeds of melon cv. Valenciano Amarelo and 30 seeds of broccoli cv. Calabres were sown per pot. After 20 days, disease severity was assessed using the scale described by Chung et al. (1988) and the respective fungi were re-isolated. In pathogenicity tests, all isolates associated with tomato caused stem rot and isolates from broccoli, spinach and melon caused hypocotyl and root rot symptoms in their respective host plant seedlings. The isolates showed different symptom severity. Isolates TO-5 and TO-3, BR-1 and BR-2, EP-2 and EP-3, ME-1 and ME-3 were more pathogenic ($P = 0.05$) to tomato, broccoli, spinach and melon, respectively (Table 2). All isolates from lettuce caused bottom rot symptoms in lettuce and isolate AL-3 was more pathogenic ($P = 0.05$) (Table 2).

The present study represents the first report, in Brazil, of the occurrence of AG 4 HG-I causing hypocotyl and root rot in melon and stem rot in tomato, of AG 4 HG-III causing hypocotyl and root rot in broccoli and spinach and, AG 1-IB causing bottom rot in lettuce.

Table 2. Virulence of isolates of *R. solani* on tomato (TO), lettuce (AL), cabbage (BR), spinach (EP), and melon (ME)

Disease severity index[1]				Disease severity index[2]					
TO-5	4.00a	AL-3	4.00a	BR-1	2.08a	EP-2	1.62a	ME-1	2.00a
TO-3	3.67ab	AL-1	3.20b	BR-2	1.98ab	EP-3	1.50ab	ME-3	1.78ab
TO-4	3.13bc	AL-2	2.67c	BR-3	1.80b	EP-1	1.36b	ME-2	1.66b
TO-2	1.76c								
TO-1	1.38d								
Control	0.00		0.00d		0.00c		0.00c		0.00c
($P = 0.05$)[3]	S		S		S		S		S

[1]Disease severity scored as 0 = no symptoms, 1 = 1–25% leaf and stem lesions, 2 = 26–50% leaf and stem lesions, 3 = 51–75% leaf and stem lesions; 4 = >75% leaf and stem lesions.
[2]Disease severity scored as 0 = no symptoms, 1 = hypocotyl and cotyledon symptoms, 2 = pre-emergence damping off, 3 = post-emergence damping off.
[3]Means scores calculated by the Tukey test ($P = 0.05$). The same letter within a column indicate significant (S) or not significantly (NS) different.

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