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Characterization of *Rhizoctonia solani* associated with soybean in Brazil

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Abstract

Rhizoctonia solani causes pre- and post-emergence damping-off, root and hypocotyl rot and foliar blight in soybean. Foliar blight has resulted in yield losses of 31–60% in north and northeast Brazil. The aim of this study was to characterize isolates of *R. solani* associated with soybean in Brazil. Among 73 *Rhizoctonia* isolates examined, six were binucleate and 67 were multinucleate. The multinucleate iso1ates were characterized according to hyphal anastomosis reaction, mycelial growth rate, thiamine requirement, sclerotia production, and RAPD molecular markers. Four isolates that caused hypocotyl rot belonged to AG-4 and using RAPD analysis they grouped together with the HGI subgroup. Another isolate that caused root and hypocotyl rots was thiamine auxotrophic, grew at 35 °C, and belonged to AG-2-2 IIIB. All 62 isolates that caused foliar blight belonged to AG-1 IA. RAPD analysis of *R. solani* AG-1 IA soybean isolates showed high genetic similarity to a tester strain of AG-1 IA, confirming their classification. The teleomorph of *R. solani*, *Thanatephorus cucumeris* was produced *in vitro* by one AG-1 IA isolate from soybean. The AG-4 and AG-2-2 IIIB isolates caused damping-off and root and hypocotyl rots of soybean seedlings cv. 'FT-Cristalina', under greenhouse conditions. The AG-2-2 IIIB isolate caused large lesions on the cortex tissue, that was distinct from the symptoms caused by AG-4 isolates. The AG-1 IA isolates caused foliar blight in adult soybean plants cv. 'Xingu' under the greenhouse and also in a detached-leaf assay.

Introduction

In Brazil, soybean is one of the most important crops, generating over US$3 billion per year (Yorinori, 1996). Diseases have been the major problem in soybean production with an estimated yield reduction of 15–20% per year (Embrapa, 1999). Between 1970 and 1998, more than 40 diseases were identified in the Brazilian soybean crop (Yorinori, 1998). This number is increasing as new areas are opened to soybean cultivation.

Rhizoctonia solani Kühn, teleomorph *Thanatephorus cucumeris* (Frank) Donk, is a destructive soilborne pathogen attacking many crops worldwide and has economic impact in all soybean-producing areas. In Brazil, *R. solani* causes pre- and post-emergence damping-off, root and hypocotyl rot, and foliar blight in soybean (Embrapa, 1999). Foliar blight has resulted in yield losses of 31–60% in north and northeast Brazil (Meyer and Yorinori, 1999). The incidence of foliar blight in Brazilian soybean fields has the potential to increase due to crop expansion into the north and northeast of the country, where high temperatures and humidity are favourable for disease development (Yorinori, 1998; Embrapa, 1999). In these regions, bean, pea, tomato, and watermelon crops are also cultivated. As alternative hosts of *R. solani*, they may constitute inoculum sources for the soybean crop (Embrapa, 1999).

Soybean rhizoctonia foliar blight (RFB) is difficult to control because labelled fungicides have a limited effect (Harville et al., 1996). However, in Brazil, alternative control methods, based on the ecology of the fungus, have been applied for *Rhizoctonia* control (Fenille and Souza, 1999). *Rhizoctonia solani* is genetically diverse. Anastomosis groups (AGs) and

AG subgroups have been used for identification and classification purposes (Parmeter et al., 1969; Ogoshi, 1987; Sneh et al., 1991; Carling, 1996). According to Carling (2000) there are 14 AGs in *R. solani:* AG-1 to AG-13 and AG-BI. Up to now, 3 subgroups of AG-1 (IA, IB, and IC), 8 of AG-2 (2-1 I, 2-1 II, 2-1 III, 2-2 IIIB, 2-2 IV, 2-2 LP, and 2-3), 3 of AG-4 (HGI, HGII, and HGIII), 2 of AG-6 (HGI and GV), 5 of AG-8 (ZG1-1, ZG1-2, ZG1-3, ZG1-4, and ZG1-5), and 2 of AG-9 (TP and TX) have been reported (Carling, 2000; Kuninaga and Carling, 2000; MacNish and O'Brien, 2000). AG characterization of isolates is carried out by observing anastomosis reaction. Isolates belong to the same AG if there is attraction and fusion of isolate hyphae; different AGs do not display this behaviour (Ogoshi, 1987). Other characteristics are also useful for *R. solani* AG and subgroup identification: thiamine requirement, optimum temperature for growth, sclerotium type, host origin, and symptoms (Ogoshi and Ui, 1979; Ogoshi, 1987). Characterization of the AGs of isolates and standard assay techniques could be important for screening soybean germplasm for disease resistance (Muyolo et al., 1993b).

Jones and Belmar (1989) verified differences between AG-1 IA and AG-1 IB regarding optimal growth temperature, fungicide sensitivity, and propagule survival conditions. Correct identification of the causal pathogen is important for appropriate selection of isolates for resistance-screening programmes and to understand the inoculum density/disease incidence relationship in rice and soybean, as well as for intercropping or crop rotation decisions. Taxonomic differences between AGs or subgroups of *R. solani* are frequently correlated to ecological and epidemiological differences (Ogoshi, 1987). Naito et al. (1995) described a soybean foliar disease caused by *R. solani* AG-2-3 in Japan. The symptoms resembled those of sugar beet leaf blight and tobacco target spot caused by *R. solani* AG-2-2 IV (Naito, 1984) and AG-3 (Shew and Main, 1990; Stevens et al., 1993), respectively, rather than those of aerial blight and web blight caused by *R. solani* AG-1 IA and IB, respectively. The differentiation between AG 2-3 and AG-1 IA and IB is not only by symptoms, but also by infection type. AG-1 IA and IB caused infection by hyphae, whereas AG 2-3 caused infection by basidiospores (Atkins and Lewis, 1954; Yang et al., 1990; Naito et al., 1995).

Throughout the world, AG-1 (IA, IB, and IC), AG-2-2 IIIB, AG-2-3, AG-4, AG-5, and AG-7 have been reported to be pathogenic to soybean (Parmeter et al., 1969; Naiki and Ui, 1981; Bolkan and Ribeiro, 1985; Jones and Belmar, 1989; Yang et al., 1990; Liu and Sinclair, 1991; Muyolo et al., 1993a; Naito et al., 1993; 1995). In Brazil, Bolkan and Ribeiro (1985) described one isolate from leaves and four isolates from hypocotyl as belonging to AG-1 and AG-4, respectively.

The experiments in this study were designed to characterize soybean isolates by hyphal anastomosis reactions, morphological and cultural characteristics, pathogenicity, and random amplified polymorphic DNA (RAPDs) to determine which AGs cause disease of soybean in Brazil. The results provide information for future ecological and epidemiological studies of *R. solani* in soybean, as well as information for breeding programmes in Brazil.

Materials and methods

Pathogen isolates

Putative *Rhizoctonia* isolates were obtained from soybean seedlings with hypocotyl rot and from adult plants with foliar blight symptoms. Isolation was achieved by cutting small sections (0.5–0.7 cm long) from advancing lesions and surface sterilizing by immersion in 70% alcohol for 30 s, 2% sodium hypochlorite for 30 s, washing in sterile distilled water and placing the tissue directly on Ko and Hora medium (Ko and Hora, 1971) containing 5 mg ml^{-1} of prochloraz (Castro et al., 1988) and 240 mg l^{-1} metalaxyl (Ceresini et al., 1996). After 24–48 h at 26 °C, colonies were examined for typical *Rhizoctonia* growth and hyphal tips from appropriate cultures were then transferred to potato-dextrose-agar (PDA) medium. The isolates were stored on PDA medium at room temperature and on rice grains at −20 °C (Table 1). Nuclear condition of isolates was determined by counting nuclei in stained vegetative cells (1 μg ml^{-1} DAPI (4′,6′-diamidino-2-phenylindole)) (Martin, 1987). Twenty cells from each isolate were examined with a fluorescent microscope (300× magnification).

Anastomosis group (AG) determination

Each isolate was paired with tester strains of *R. solani*, obtained from researchers worldwide. The testers were of subgroups described previously from soybean (Parmeter et al., 1969; Naiki and Ui, 1981; Bolkan and Ribeiro, 1985; Jones and Belmar, 1989; Yang et al., 1990; Liu and Sinclair, 1991; Muyolo et al., 1993a; Naito et al., 1993; 1995). Pairing methodology

Table 1. Origin and hyphal anastomosis groups of *Rhizoctonia* spp. isolates from Brazil

Isolate	Symptoms	Origin/year	Anastomosis group
SJ 01	Hypocotyl rot	Minas Gerais/ 1994	AG-4
SJ 02	Hypocotyl rot	Paraná/1996	AG-4
SJ 03	Hypocotyl rot	Paraná/1996	AG-4
SJ 05	Hypocotyl rot	São Paulo/1997 (Area 1*)	AG-4
SJ 06	Hypocotyl rot	São Paulo/1997 (Area 1)	Binucleate
SJ 07	Hypocotyl rot	São Paulo/1997 (Area 1)	AG-2-2 IIIB
SJ 08	Hypocotyl rot	São Paulo/1997 (Area 1)	Binucleate
SJ 10	Hypocotyl rot	São Paulo/1997 (Area 2)	Binucleate
SJ 11 and 12	Hypocotyl rot	São Paulo/1997	Binucleate
SJ13 to 16 and 19–22	Foliar blight	Mato Grosso/ 1998 (Area 1)	AG-1 IA
SJ 23, 24 and 26–31	Foliar blight	Mato Grosso/ 1998 (Area 2)	AG-1 IA
SJ 33, 34, 36–54 and 56–80	Foliar blight	Mato Grosso/ 1998 (Area 3)	AG-1 IA
SJ 81	Hypocotyl rot	São Paulo/1998 (Area 3)	Binucleate

SJ = soybean. *Different fields in the same region.

for AG determinations was according to Ceresini et al. (1996). Anastomosis was regarded as positive when hyphae grew together, made contact, and their walls fused, with subsequent death of adjacent cells. Hyphal anastomosis was observed with light microscopy after staining the vegetative cells with 0.03% safranine-O aqueous solution and 3% KOH aqueous solution (Yamamoto and Uchida, 1982).

Characterization of the anamorph

The various field isolates were grown on PDA amended with 50 mg l^{-1} of oxytetracycline, incubated at 28 °C for 28 days and then assessed for sclerotia size and shape, and colony morphology. Radial growth rate (mm/day) of the isolates was measured after 48-h dark incubation. Isolates were grown at 5–40 °C on PDA in 5 °C increments. A completely randomized design with five replicate plates per isolate was used.

Thiamine requirement

Thiamine requirement of the 16 foliar blight and five hypocotyl rot isolates was determined by methods described by Ogoshi and Ui (1979) and Stevens-Johnk and Jones (1993). One AG-1 IA (prototrophic), one AG-2-2 IIIB (auxotrophic) and two AG-4 (prototrophic) tester isolates were included as controls. A completely randomized design with four replications per isolate was used. The thiamine requirement of each isolate was determined by the relationship (ratio B/A) between the weight of dried mycelia produced with (B) and without thiamine (A) in glucose asparagine medium (Liu and Sinclair, 1991; Carling et al., 1994).

Teleomorph induction and morphology

To promote teleomorph formation, a mycelial suspension (10 : 1 water : mycelia) of one *R. solani* soybean isolate causing foliar blight was inoculated onto detached and rooted soybean trifoliate leaves. The inoculated leaves were kept in a humid glass chamber at 28 ± 1 °C and 12-h photoperiod. The morphology of basidia, sterigmata, basidiospores, supporting hyphae of the basidium, and hyphal branching of the hymenium tissues were examined under light microscopy (100–400× magnification), using lactophenol–cotton blue as a mounting stain.

RAPD analysis

The *R. solani* isolates from soybean and the tester isolates were grown in 200 ml potato-dextrose broth for 7 days at 26 °C in the dark. DNA was isolated as described by Kuramae-Izioka (1997). All soybean multinucleate isolates and the testers of AG-1 IA, IB, and IC; AG-2-1; AG-2-2 IIIB; AG-2-2 IV; AG-2-3; AG-4 HGI and HII were analyzed by RAPDs using different primers (OPA-09, OPA-13, OPP-03, OPP-10, OPP-11, OPP-12, OPP-14, OPP-15, OPP-17, OPP-18, and OPP-19; Operon Technologies Inc., Alameda, CA) essentially as described by Williams et al. (1990). Negative controls, in which DNA template solution was replaced by sterile water, were included in all experiments to test for contamination. After amplification, samples were separated on a 1.5% (w/v) agarose gel, stained with ethidium bromide and photographed under UV light. Only strongly stained bands were considered for analysis. Comparison of each profile was carried out based on the presence (1) or absence (0) of amplified products of the same length. Bands of the same length were scored as being identical. Analyses were based on the Simple Matching Coefficient. The dendrogram was derived from the distance matrix by the Unweighted Pair-Group Method Arithmetic Average (UPGMA) using the NTSYS-pc 1.7 (Numerical Taxonomy and Multivariate Analysis System) computer program

(Rohlf, 1992). In addition, bootstrap analysis was carried out using the PAUP* program (Phylogenetic Analysis Using Parsimony, *and other methods) (Swofford, 1998) to determine the robustness of the cluster.

Pathogenicity test

The isolates were tested on soybean cv. 'FT-Cristalina' in greenhouse conditions at 25 ± 2 °C, in soils at pH 4.5 or 5.9. Inoculum was prepared as described previously (Fenille and Souza, 1999). A completely randomized design with five replicate pots per isolate was used. After 15 days, disease severity was assessed using the scale described by Davey and Papavizas (1959) and Fenille and Souza (1999). All isolates derived from plants with foliar blight were evaluated for pathogenicity. Whole plant assays were carried out in greenhouse experiments on 41-day-old plants at 25 ± 2 °C (Bolkan and Ribeiro, 1985). An *in vitro* detached-leaf assay was carried out using healthy leaves of 55-day-old soybean plants at 26 ± 1 °C (Muyolo et al., 1993a). The soybean cultivar used in both tests was 'Xingu'.

Results

Nuclear number. Among the eleven isolates of *Rhizoctonia* obtained from soybean seedlings showing hypocotyl rot, five were multinucleate and six were binucleate. All 62 isolates obtained from soybean plants with foliar blight symptoms were multinucleate (Figure 1A). The binucleate isolates contained 1.8–2.3 nuclei/cell while the multinucleate isolates contained 8–23 nuclei/cell.

Anastomosis group determination. Among the five multinucleate isolates from plants with root and hypocotyl rots, four anastomosed with the AG-4 tester and one with the AG-2-2 IIIB tester. All multinucleate isolates from plants with foliar blight symptoms anastomosed with the tester of AG-1 IA (Figure 1B).

Anamorph morphology. The four isolates which anastomosed with the AG-4 tester displayed light-brown to brown mycelium on PDA medium. Young sclerotia were small and light-brown in colour; mature sclerotia were brown. These isolates grew at temperatures ranging from 10 to 35 °C. The maximum growth was 15.8–19.0 mm day^{-1} at 25 and 30 °C, respectively. The isolate that anastomosed with the AG-2-2 IIIB tester grew at temperatures ranging between 10 and 35 °C. Maximum growth was 14.9 mm day^{-1}at 25 °C. This isolate produced small, irregular shaped, light-brown mature sclerotia on PDA. Isolates associated with foliar blight on soybean plants showed light-brown mycelium on PDA and produced a large number of 1–3 mm dia sclerotia (Figure 1D). The sclerotia were whitish and dark-brown/black at early and maturity stages, respectively. Exudation of dark-brown drops was frequently observed on the surface of the sclerotia. These isolates showed similar mycelial growth at temperatures ranging from 10 to 35 °C. Maximum growth was at temperatures between 25 and 30 °C with rates of 13.4–22.8 mm day^{-1}, respectively. These isolates were morphologically similar to three Japanese isolates of AG-1 IA (Figure 1E) obtained from maize and rice plants. These isolates grew on average 23 mm day^{-1}.

Thiamine requirements. All isolates that anastomosed with the AG-4 and AG-1 IA testers were prototrophic for thiamine, similar to the testers of subgroups AG-4 HGI, AG-4 HGII, and AG-1 IA from the USA and Japan. The B/A ratio of the isolates that anastomosed with the AG-4 tester was 0.8–0.9; for isolates associated with soybean foliar blight the B/A ratio was 0.5–2.2. The isolate that anastomosed with the AG-2-2 IIIB tester was auxotrophic for thiamine and its B/A ratio was 8.0, higher than the ratio of the AG-2-2 IIIB tester from the USA.

Teleomorph induction and morphology. Ten days after inoculation, white hymenia were observed on the surface of the glass chamber where the inoculated leaves were kept. Basidia and basidiospores were observed three weeks after inoculation (Figure 1C). These basidia were barrel- to subcylindrical-shaped, 11 × 5.9 μm in size. Typical basidia were 1.6 times as wide as the supporting hyphae and produced 2–5 variable-length sterigmata of up to 4.7 μm. Basidiospores were hyaline and ellipsoid to ovoid (4.4 × 3.6 μm).

RAPD analysis. Thirty-five polymorphic RAPD bands were produced by the soybean multinucleate isolates and the AG-1 IA, AG-2-2 IIIB, and AG-4 HGI testers. Three distinct groups were distinguishable. The first group contained the four isolates from soybean hypocotyl rots and tester AG-4 HGI. The second group combined 62 isolates associated with soybean foliar blight and tester AG-1 IA. The third group contained one soybean isolate and tester AG-2-2 IIIB (data not shown). These three groups

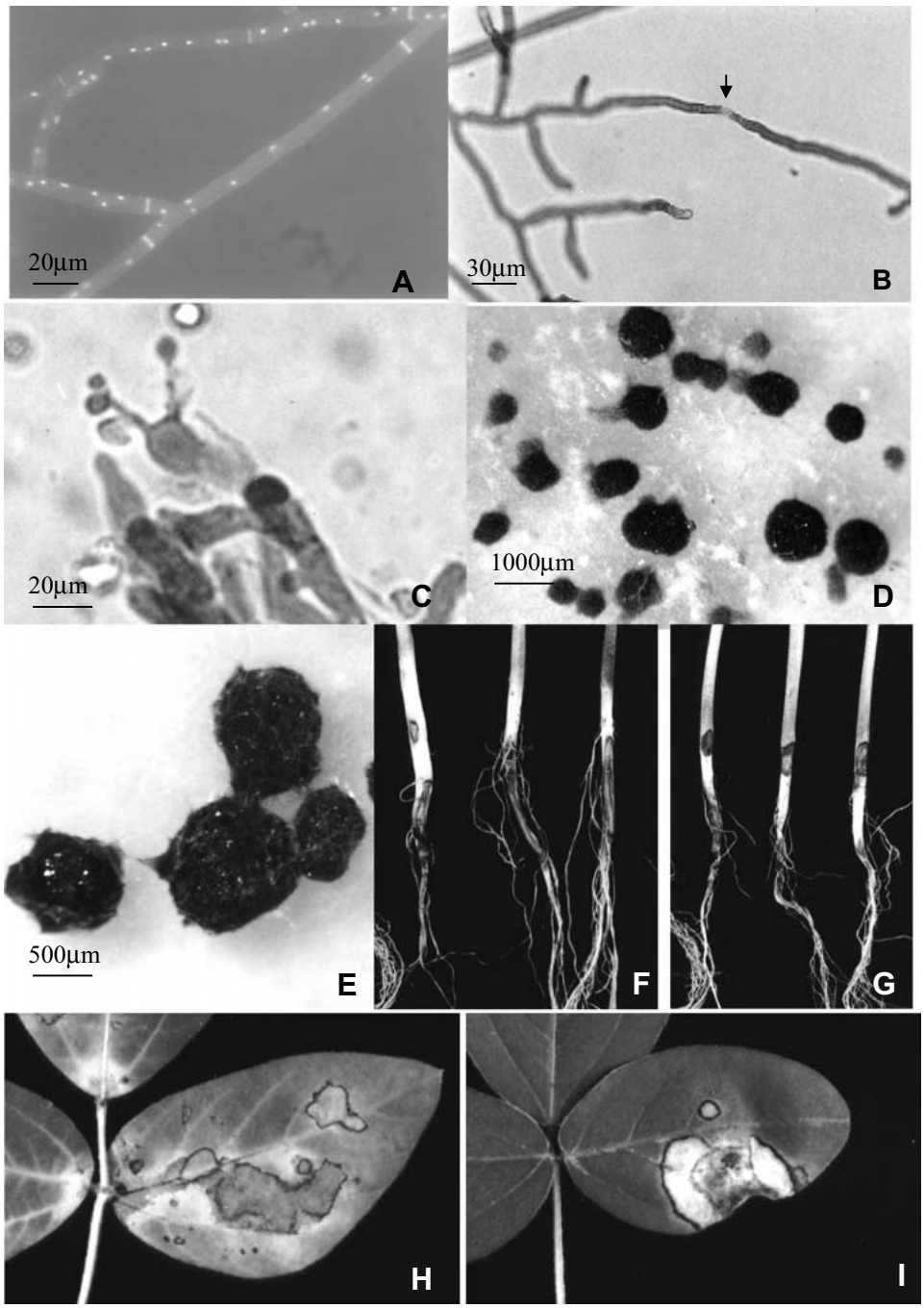

Figure 1. Morphological and pathogenic characteristics of *Thanatephorus cucumeris* isolates (anamorph *Rhizoctonia solani*). A: multinucleate cells; B: hyphal anastomosis (arrow) of a Brazilian soybean isolate with the tester isolate of AG-1 IA; C: basidium and young basidiospores; D and E: sclerotial type sasakii produced by a Brazilian AG-1 IA isolate (D) and a Japanese AG-1 IA isolate (E); F: infection of soybean seedlings by a Brazilian AG-2-2 IIIB isolate; G: infection of soybean seedlings by a Brazilian AG-4 isolate; H and I: infection of soybean leaves by a Brazilian AG-1 IA isolate in detached-leaf (H) and greenhouse (I) assays.

were analyzed separately. A total of 23 polymorphic fragments was observed among the AG-4 group. The four soybean isolates were most closely related to the AG-4 HGI tester (Figure 2A). The isolate that anastomosed with the USA soybean isolate of AG-2-2 IIIB was compared with testers representing AG-2-1, AG-2-2 IIIB, AG-2-2 IV, and AG-2-3. Thirty-nine polymorphic fragments were obtained. The Brazilian soybean isolate SJ07 clustered together with the AG-2-2 IIIB tester isolate (Figure 2B). Among the 62 isolates associated with foliar blight, ten were compared to subgroup testers AG-1 IA (from Japan), and IB and IC (from the USA). Twenty-three polymorphic fragments were obtained. The Brazilian isolates were most closely related to the AG-1 IA tester, while similarity between these isolates and AG-1 IB and AG-1 IC, which grouped together was less than 0.4 (Figure 2C).

Pathogenicity tests. The five soybean isolates associated with hypocotyl rot symptoms caused root and hypocotyl rot on soybean seedlings in the greenhouse (Figure 1F and G). No significant differences were observed among the isolates ($P = 0.05$) at pH 4.5 but at pH 5.9 the soybean isolate AG-2-2 IIIB was less aggressive than the AG-4 isolates. At pH 5.9, the disease index of the isolate AG-2-2 IIIB was 1 (0–7 scale), not statistically different from the control ($P = 0.05$). All isolates associated with soybean foliar blight caused foliar lesions on soybean plants in greenhouse and detached-leaf assays (Figure 1H and I), but symptom severity varied widely between isolates. Four of the 62 isolates

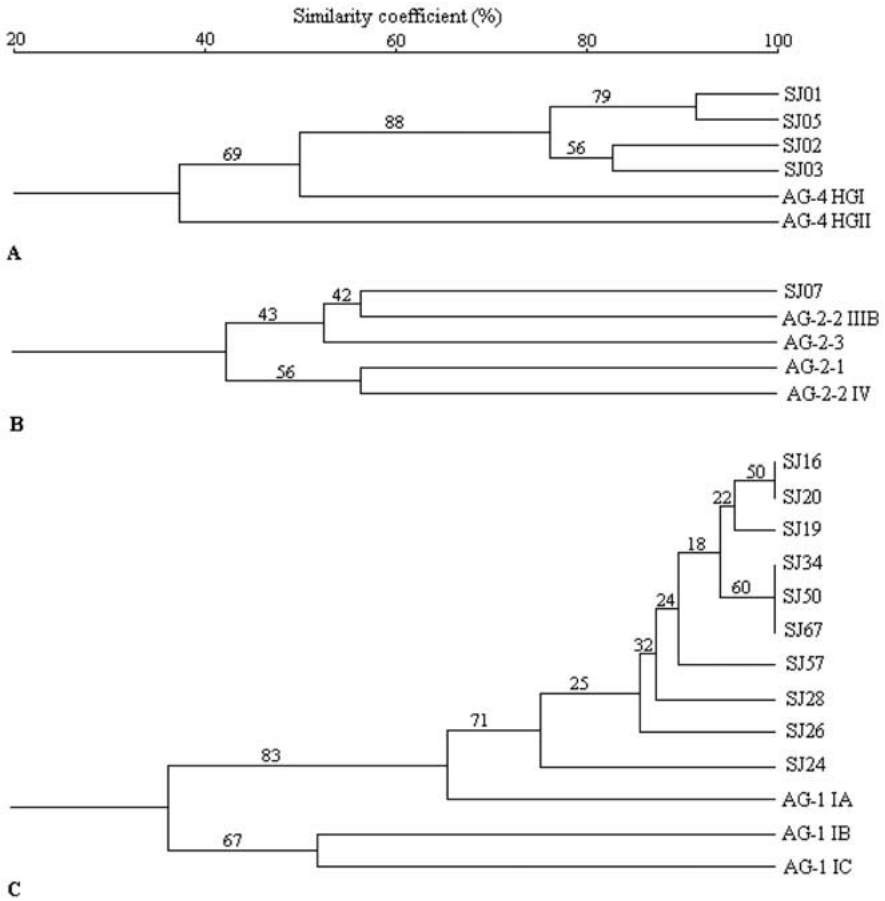

Figure 2. Dendrograms obtained from similarity coefficients after UPGMA–SAHN clustering of band data generated by RAPDs of *Rhizoctonia solani* from soybean (Table 1) and *Rhizoctonia solani* testers (SJ = soybean). Primers utilized: (A) OPA-09, OPA-13, OPP-14, OPP-15, and OP-17; (B) OPA-13, OPP-12, OPP-17, and OPP-15; and (C) OPA-09, OPA-13, OPP-12, OPP-14, and OPP-17. Bootstrap values obtained using the PAUP program are written on the cluster branches.

were significantly more aggressive under both inoculation conditions. The highest average disease index was 2.1 in the greenhouse experiments and 3.2 in the detached-leaf assay, on a 0–4 scale.

Discussion

In Brazil, isolates of *R. solani* are associated with three types of symptoms on soybean. According to Embrapa (1999), these symptoms are damping-off, root and hypocotyl rot, and foliar blight. This study was also able to determine the impact of climatic conditions on the symptoms in soybean. In the States of Paraná, São Paulo and Minas Gerais at latitudes more than 15°S, *Rhizoctonia* spp. are present causing damping-off and root and hypocotyl rot, while in regions of Mato Grosso State, at latitudes from 0°S to 15°S with high temperature and humidity there is an association with soybean foliar blight. High temperature and humidity are necessary for foliar blight to occur. The occurrence of damping-off, and root and hypocotyl rots is limited by the chemical treatment of seeds, crop rotation, and organic debris management which explains the low number of isolates obtained in this study (Table 1).

Among the 73 isolates of *Rhizoctonia* spp. obtained in this study, 8% were binucleate. These isolates were exclusively obtained from seedlings with hypocotyl rot symptoms. Binucleate isolates were generally not pathogenic to soybean seedlings by *in vitro* inoculation tests (data not shown). The association of non-pathogenic binucleate *Rhizoctonia* with diseased plants also has been observed by Ceresini et al. (1996) and Ceresini and Souza (1997) in Brazil, in peanut and bean, respectively. According to Parmeter et al. (1969), Liu and Sinclair (1991), Muyolo et al. (1993a,b), and Naito et al. (1993), symptoms of damping-off, and root and hypocotyl rot in soybean are caused by AG-1 IC, AG-2-2 IIIB, AG-4, and AG-7 of *R. solani.* In this study, four isolates causing these symptoms belonged to AG-4 and one belonged to AG-2-2 IIIB. In our study, anastomosis reactions, phenotypic characters and pathogenicity did not separate the two AG-4 subgroups (HGI and HGII). According to Exner (1953) and Sherwood (1969), culture medium and isolate temperature maintenance can change the morphology of *R. solani*. The separation of the two subgroups was verified by Kuninaga and Yokosawa (1984) by combining different characters such as DNA base sequence homology, electrophoretic patterns of proteins, cultural appearance, mycelial growth rate, and pathogenicity. When RAPD molecular markers were utilized, we observed that the four soybean isolates grouped the best with AG-4 HGI.

Hyphal anastomosis is sufficient for grouping the soybean *R. solani* to AG-2-2. According to Ogoshi (1987), fusion between isolates of AG-2-1 with those of AG-2-2 is infrequent. Specific characteristics, such as thiamine requirement and temperature, could separate the AG-2-2 subgroups, AG-2-2 IIIB and AG-2-2 IV. The one Brazilian isolate was auxotrophic to thiamine, a characteristic of AG-2-2, and it grew at 35 °C, characteristic of the AG-2-2 IIIB subgroup. In contrast, the tester of AG-2-2 IV did not grow at 35 °C, as also observed by Hyakumachi et al. (1998). In general, AG-4 isolates were more aggressive than that of AG-2-2 IIIB. However, at pH 4.5 all the isolates were considerably aggressive with a disease index at 3.4–6.0 (0–7 scale). The AG-2-2 IIIB isolate infected the roots, essentially, while AG-4 isolates caused lesions on the hypocotyls. Muyolo et al. (1993a) also observed in pathogenicity tests that AG-2-2 IIIB isolates were more aggressive in roots than hypocotyls, while AG-4 isolates were more aggressive in hypocotyls than in roots. The symptoms caused by the AG-2-2 IIIB isolate correspond to those described by Liu and Sinclair (1991) causing soybean root rot (Illinois, USA) and differ from symptoms observed by AG-4 isolate infection. In the seedlings inoculated with the AG-2-2 IIIB isolate, the lesions were larger than those caused by AG-4 isolates; also cortex tissue and root fading were seen in the plant inoculated with the AG-2-2 IIIB isolate (Figure 1F). Lesions caused by the AG-4 isolate were delimited, sunken, and with a dark border, but without cortex tissue exhibition (Figure 1G).

Two isolates, one characterized as AG-4 and the other as AG-2-2 IIIB were obtained from the same soybean field where crop rotation of bean–wheat–soybean is common. These two AGs have also been reported by Ceresini and Souza (1997) associated with beans in different regions of São Paulo State. This highlights the importance of knowledge about *R. solani* AGs in different crops, mainly when deciding what to cultivate next.

We could not conclude by hyphal anastomosis alone that the soybean isolates causing foliar blight belonged to AG-1 IA. However, characteristics such as mycelial growth rate and sclerotium type helped with AG identification. The growth curve of isolates causing foliar blight and the maximum mycelial growth rate was similar to isolates representing AG-1 IA and higher than the AG-1 IB isolate, another subgroup of AG-1 that

also can cause foliar blight in soybean (Yang et al., 1990). Another important characteristic for identifying subgroups of AG-1 is the type of sclerotia produced. All isolates associated with soybean foliar blight in Brazil produced large round sclerotia, characterized as sasakii according to Jones and Belmar (1989). These isolates and the AG-1 IA tester were prototrophic to thiamine with B/A ratio ranging from 0.5 to 2.2. These results corroborate those obtained with other AGs such as AG-9, whose ratio of 3.1 was used to characterize isolates as prototrophic; only ratios above 5.0 determined isolates as belonging to the same AG and being auxotrophic to thiamine (Yang et al., 1996).

Production of basidia and basidiospores by an AG-1 IA soybean isolate (SJ68, Table 1) confirmed the occurrence of the teleomorphic phase of *R. solani*, *T. cucumeris*. The dimensions, number and length of sterigmata were lower than previously described sexual structures of *T. cucumeris* of other AGs (Carling et al., 1987; 1994). This may be due to the number of samples measured from only one isolate. Attempts in this study to induce basidiospore formation *in vitro* using the techniques of Tu and Kimbrough (1975), Hyakumachi and Ui (1988), Kangatharalingam and Carson (1988), and Naito et al. (1995) were unsuccessful. In plants with foliar blight symptoms from which the isolates were obtained, no evidence of sexual structures was found. This could be because of the epidemiological character of AG-1 IA; according to Naito et al. (1995), AG-1 IA infection is through the hyphae. However, the occurrence of the teleomorph in Brazilian soybean fields should be studied. According to Pascual et al. (2000), in regions of the Philippines with high temperatures and humidity throughout the year, AG-1 IA basidiospores are considered an important source of inoculum.

RAPD analysis was important in the confirmation of AG-1 IA isolates causing soybean foliar blight in Brazil. The high genetic similarity between isolates is probably due to a common geographic origin. This agrees with Pascual et al. (2000) that isolates from AG-1 IA obtained from different hosts, but collected from the same region, showed more than 80% genetic similarity. According to Jabaji-Hare et al. (1990) in the same AG of *R. solani* there is more genetic similarity among isolates from a common geographic origin than among isolates from different geographic areas. The 67% genetic similarity between Brazilian soybean isolates causing foliar blight and the Japanese AG-1 IA tester obtained from maize, is similar to data obtained from AG-1 IA isolates of mung bean, bluegrass, cogon (*Imperata cylindrica*), maize, itch grass (*Rottboellia exaltata*), coffee, cowpea, sugarcane, rice, and sorghum in which the similarity was 75% (Pascual et al., 2000).

In Louisiana (USA), Yang et al. (1990) reported the occurrence of *R. solani* AG-1 IA and IB causing lesions in leaves of soybean. AG-1 IB was predominant in these areas during the two-year period studied. Climatic differences between North and South America could be a factor determining which AG-1 subgroup dominates as causal organisms of foliar blight in soybean.

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