

Rhizoctonia solani AG-1 IA infects both rice and signalgrass in the Colombian Llanos¹

Lina Maria Ramos-Molina², Edison Chavarro-Mesa², Danilo Augusto dos Santos Pereira³, María del Rosario Silva-Herrera⁴, Paulo Cezar Ceresini³

ABSTRACT

Foliar blight and death of signalgrass (*Urochloa* spp.) pastures are caused by the *Rhizoctonia solani* fungus. This study aimed at determining which pathogens from the *Rhizoctonia* species complex are associated with leaf and sheath blight in *Urochloa* and rice, in the Colombian Llanos. Sympatric areas of *Urochloa* pastures adjacent to rice cropping areas were sampled using a linear transect system. The pathogens were identified using morphological traits, molecular detection based on specific primers and sequencing of the ITS-5.8S rDNA region. *R. solani* AG-1 IA predominated as the pathogen associated with foliar blight in all samples from *U. brizantha* cv. 'Toledo' and hybrid *Urochloa* cv. 'Mulato'. Besides *R. solani* AG-1 IA (18 % of the samples), *Rhizoctonia oryzae-sativae* (71 %) and *Sclerotium hydrophilum* (11 %) were also detected. In the cross-pathogenicity test, the *R. solani* AG-1 IA fungus was the most aggressive to *Urochloa*, while *R. oryzae-sativae* produced very mild infection symptoms. This is the first report of *R. oryzae-sativae* and *S. hydrophilum* associated with the complex of rice sheath blight diseases in Colombia.

KEY-WORDS: *Urochloa* spp.; *Rhizoctonia oryzae-sativae*; *Sclerotium hydrophilum*.

INTRODUCTION

Signalgrass (*Urochloa* spp.) foliar blight and collar rot diseases are caused by the Basidiomycetes *Rhizoctonia solani* (sexual stage *Thanatephorus cucumeris*) fungus, which emerged early in 1990 as an important pathogen for *Urochloa* pastures in the eastern Colombian Llanos (Ciat 1993, Argel et al. 2005, Alvarez et al. 2013). The emergence of these diseases was first reported in areas previously occupied by rice, a highly susceptible host (Lee &

RESUMO

Rhizoctonia solani AG-1 IA infecta arroz e braquiária nos Llanos Colombianos

A queima da folha e morte de pastagens do gênero *Urochloa* são causadas pelo fungo *Rhizoctonia solani*. Objetivou-se determinar que patógenos do complexo de espécies do gênero *Rhizoctonia* estão associados à queima da folha e bainha em *Urochloa* e arroz, nos Llanos Colombianos. Utilizando-se o sistema de transectos lineares, foram amostradas áreas de pastagens de *Urochloa* simpátricas, adjacentes às de arroz. A identificação dos patógenos foi efetuada por caracterização morfológica, detecção molecular baseada em iniciadores específicos e sequenciamento da região ITS-5,8S do rDNA. *R. solani* AG-1 IA predominou como patógeno associado à queima da folha em todas as amostras de *U. brizantha* cv. 'Toledo' e híbrido *Urochloa* cv. 'Mulato'. Além de *R. solani* AG-1 IA (18 % das amostras), *R. oryzae-sativae* (71 %) e *Sclerotium hydrophilum* (11 %) também foram detectados. No teste de patogenicidade, o fungo *R. solani* AG-1 IA foi o mais agressivo a *Urochloa*, enquanto *R. oryzae-sativae* ocasionou sintomas muito leves de infecção. Este é o primeiro relato de que os fungos *R. oryzae-sativae* e *S. hydrophilum* estão associados ao complexo de doenças da bainha do arroz, na Colômbia.

PALAVRAS-CHAVE: *Urochloa* spp.; *Rhizoctonia oryzae-sativae*; *Sclerotium hydrophilum*.

Rush 1983). In the same Colombian region, roughly two decades ago, the occurrence of sheath blight caused by *R. solani* AG-1 IA in rice was also observed for the first time (Pabón Guerrero 1994). *R. solani* is a complex species composed of various anastomosis groups (AGs) with high host specificity (Adams 1988, Sneh et al. 1996).

Although no precise data are available regarding economic losses caused by foliar blight and death of *Urochloa*, the disease is considered severe (Argel et al. 2005, Duarte et al. 2007, Alvarez

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2. Universidade Estadual Paulista, Departamento de Fitossanidade, Engenharia Rural e Solos, Ilha Solteira, SP, Brazil. E-mails: limaramo@gmail.com, bioedicha@gmail.com, paulo.ceresini@bio.feis.unesp.br.

3. ETH Zurich, Institute of Integrative Biology - Plant Pathology, Zurich, Switzerland. E-mail: danilo455@gmail.com.

4. Universidad de los Llanos, Villavicencio, Meta, Colombia. E-mail: rosariosilvasa@yahoo.com.

et al. 2013). The estimate of losses in rice production caused by sheath blight can reach up to 50 % (Savary et al. 2000).

In the south of the United States, *Urochloa* spp. was reported as host for two distinct AGs of the *R. solani* species complex: AG-1 IA and AG-1 IB (Black et al. 1996). A recent description from 2013 indicated a prevalence of *R. solani* AG-1 IA infecting pastures of *Urochloa* (69 % of total samples) in the warm regions of Colombia: Casane and Córdoba. On the other hand, *Rhizoctonia* sp. AG-D (*Ceratobasidium* sp.) was more commonly found in regions with lower temperatures such as Cauca (31 % of the samples) (Alvarez et al. 2013).

It is possible that different pathogens from the *Rhizoctonia* complex could be associated with foliar blight and death of *Urochloa* in the Colombian Llanos, especially in areas close to rice fields.

Besides *R. solani* AG-1 IA, in South America, the species *R. circinata* var. *oryzae*, *R. circinata* var. *zeae*, *R. circinata* var. *circinata* (sexual stage *Waitea circinata*), *R. oryzae-sativae* (sexual stage *Ceratobasidium oryzae-sativae*) and even *Sclerotium* (*S. hydrophilum* and *S. oryzae*) have already been described as being associated with the rice sheath blight complex (Cedeño et al. 1996, Madariaga et al. 1999, Gutiérrez 2007).

The management of *Urochloa* foliar blight and collar rot and rice sheath blight complexes relies heavily on varietal resistance (Nunes et al. 2004). Therefore, it is important to understand the relative importance of each *Rhizoctonia* species/groups, in order to improve disease management.

This study aimed at determining which pathogens from the *Rhizoctonia* species complex are associated with foliar and sheath blight in *Urochloa* spp. and rice, in the Colombian Llanos.

MATERIAL AND METHODS

Population samples of infected *Urochloa* and rice were collected between 2010 and 2011, in the Meta State, eastern Colombian Llanos, in Colombia (Figure 1). Samples were collected in Puerto López, from *Urochloa brizantha* cv. 'Toledo' (BBT1) and hybrid *Urochloa* cv. 'Mulato' (BHM3) with foliar blight symptoms, and in adjacent areas from 'Fedearroz 50' (OS5) rice plants with sheath blight or spot symptoms (Figure 1, Table 1).

The transect sampling system, with six lines in each cropping area and seven to eight sampling points per line, was used. Employing the same system, the following hosts with disease symptoms were sampled in the Villavicencio county: *U. brizantha* cv. 'Toledo' (BBT2), hybrid *Urochloa* cv. 'Mulato' (BHM4) and rice cv. 'Thailandia' (OS6) (Figure 1, Table 1). Isolations were initially performed by disinfecting leaf fragments in sodium hypochlorite 1 %, followed by transference to PDA media (potato-dextrose-agar,

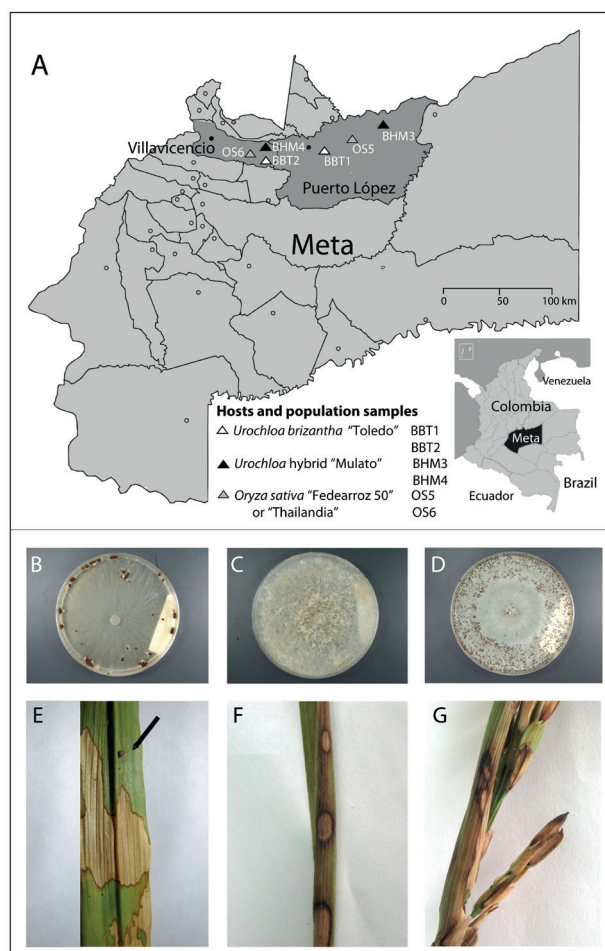


Figure 1. Geographic locations of *Urochloa* spp. and rice sampled in Colombia, morphological differences among fungi and the disease symptoms of foliar blight and sheath spot or blight. (A) Geographic location of sampled areas close to Puerto López and Villavicencio, in Meta; (B) sasakii-type sclerotia of *Rhizoctonia solani* AG-1 IA; (C) sclerotia of *R. oryzae-sativae*; (D) sclerotia of *Sclerotium hydrophilum*; (E) foliar blight symptoms in *Urochloa brizantha* cv. 'Toledo' related to *R. solani* AG-1 IA; (F and G) symptoms of rice sheath blight related to *R. oryzae-sativae* and *Sclerotium hydrophilum*.

Table 1. Populations of *Urochloa* spp. and rice associated with foliar fungi pathogens.

District	Host	Cultivar	Population	Sample size (N) ^a	Total isolate number ^b		
					<i>Rhizoctonia solani</i> AG-1 IA	<i>R. oryzae-sativae</i>	<i>Sclerotium hydrophilum</i>
Puerto López	<i>U. brizantha</i>	‘Toledo’	BBT1	38	38	-	-
	<i>Urochloa</i>	Hybrid ‘Mulato’	BHM3	40	40	-	-
	Rice	‘Fedearroz 50’	OS5	38	2	36	-
Villavicencio	<i>U. brizantha</i>	‘Toledo’	BBT2	42	42	-	-
	<i>Urochloa</i>	Hybrid ‘Mulato’	BHM4	41	41	-	-
	Rice	‘Thailandia’	OS6	35	11	16	8
Total				234	174	52	8

^a N = sample size (number of fungi isolates obtained from symptom association in each sampled species); ^b characterization based upon PCR detection.

Himédia) with 50 µg mL⁻¹ of chloramphenicol and streptomycin. Plates were kept at 25 °C in the dark for up to 48 h. Pure cultures were isolated after transference of typical *Rhizoctonia*-like mycelia to new PDA plates.

For DNA extraction, fungal mycelium was grown in 30 mL of potato dextrose broth (18.5 g L⁻¹), for 5 days, on a shaker at 75 rpm, after which the mycelium was collected by filtration and freeze-dried for approximately 48 h. DNA was extracted using a GenElute kit (Sigma-Aldrich), according to the manufacturer’s instructions.

Isolates were identified based upon mycelia and sclerotia morphological characteristics (Sneh et al. 1996, Yang et al. 1989, Costa-Souza et al. 2007, Gutiérrez 2007, Lanoiselet et al. 2007) and via polymerase chain reaction (PCR), using primers for the ribosomal DNA regions 28S and ITS-5.8S (Johanson et al. 1998, Matsumoto 2002). Selective amplification of the 28S rDNA was performed for all isolates using RS-CMF/AG-1AR primer pairs specific to *R. solani* AG1-IA (Matsumoto 2002). For rice isolates, primers GMROS-6/R635 for the ITS-5.8S rDNA, specific to *R. oryzae-sativae*, were used (Johanson et al. 1998). PCR conditions were the same as described previously (Johanson et al. 1998, Matsumoto 2002).

The ribosomal DNA ITS-5.8S of 64 sampled fungi was sequenced using the primers ITS4 and ITS5 (Johanson et al. 1998, Matsumoto 2002). PCR product sequence was performed by Macrogen (South Korea). Sequence data from both ends of each amplicon (one forward and one reverse per isolate) were assembled, aligned and concatenated using Chromas (Technelysium, Australia). Phylogenetic analyses were performed through neighbor-joining (NJ) inference and the Kimura model for genetic

distance using MEGA v.5.0 (Tamura et al. 2011). The statistical support for each clade in the obtained tree was tested using bootstrap resampling with 1,500 permutations.

For the cross-pathogenicity study, 24 *R. solani* AG-1 IA isolates were selected, 12 from *Urochloa* spp. and 12 from rice. Nine *R. oryzae-sativae* isolates and seven *S. hydrophilum* isolates from rice were also included, totaling 40 isolates. The rice cultivar ‘Fedearroz’ and *U. brizantha* cv. ‘Toledo’ were used as hosts, and uninoculated control plants were included in the assay. Seeds were surface-sterilized with 1 % sodium hypochlorite for 20 min prior to sowing. Four seeds were sown in each pot filled with a soil and sand mix (2:1). After seedling emergence, the pots were thinned to one seedling per pot. Plants were fertilized with 1 g of NPK (15:15:15) granular fertilizer. The pots were watered daily and kept in a greenhouse under natural conditions until inoculation.

Inoculation with *R. solani* sclerotia, *R. oryzae-sativae* or *S. hydrophilum* mycelium plug (6 mm) was performed when the plants had four leaves. The inoculum was applied to the base of the last or second-to-last leaf of the main tiller and attached with parafilm. The infected plants were kept in a phytotron in high humidity (95 %), with daytime temperatures maintained between 25 °C and 27 °C. Evaluation was performed 6 days after inoculation of rice and 12 days after inoculation of *Urochloa* by measuring the maximum tiller length and the lesion length on the tiller. A disease index was calculated according to the following equation: 9 x (length of the lesion on the tiller/maximum tiller length) (Jia et al. 2007). The rating of the experimental unit was the combined scores from up to four tillers. To detect evidence of host specialization, analysis of variance followed by a

priori contrast analysis between groups of isolates was performed using SAS 9.1 (SAS System for Windows, SAS Institute, Cary, NC). The statistical analyses were performed independently for each experiment, being completely randomized, with three replications each.

RESULTS AND DISCUSSION

The 234 isolates obtained are described in Table 1. Based on cultural and morphological characteristics, isolates from six plant populations with visual symptoms of foliar blight and death of *Urochloa* and rice sheath spot and blight were classified in three distinct groups (Table 1). Isolates in the first group showed sasaki-type sclerotia on light brown-colored cultures (Figure 1B), similar to *R. solani* AG-1 IA (Sneh et al. 1996, Yang et al. 1989). This group contains 161 isolates from *Urochloa* spp. and 13 from rice plants.

A similar report was made by Alvarez et al. (2013), who found that *R. solani* AG-1 IA was the predominant pathogen associated with foliar blight in *U. brizantha*, *U. brizantha* cv. 'Toledo', *U. decumbens*, *U. mutica*, *Urochloa* cv. 'Mulato' and 'Mulato II', in Colombia. The second group includes 52 isolates obtained only from rice, with small and irregular-shaped sclerotia and with light brown mycelium (Figure 1C), similar to *R. oryzae-sativae* (Lanoiselet et al. 2007). The last group consists of eight isolates obtained from rice, showing small round sclerotia with a dark brown to black color (Figure 1D), like *S. hydrophilum* (Gutiérrez 2007).

The 161 fungi isolates obtained from symptomatic *Urochloa* spp. and 13 isolates obtained from sheath lesions in rice resulted in positive amplification with primers specific to *R. solani* AG-1 IA. Another 52 isolates from rice plants were positively amplified with primers specific to *R. oryzae-sativae*, and the remaining eight isolates from the third group did not exhibit amplification for any of the *Rhizoctonia* specific molecular primers used.

Phylogenetic inferences were based on rDNA ITS sequences of isolates from the three groups (Figure 2). From the first group, 55 isolates (represented by 9 distinct haplotypes in the superior phylogenetic tree) had similar sequences to *R. solani* AG-1 IA sequences. These isolates are composed of individuals from the populations BBT1 and BBT2 (15 isolates), BHM3 and BHM4 (29 isolates), and

OS5 and OS6 (11 isolates). The second biggest group, whose sequences were similar to *R. oryzae-sativae*, was composed of four isolates from the OS5 and OS6 populations. Finally, a third group of five isolates from the OS5 and OS6 populations showed similar sequences to *S. hydrophilum*. The phylogenetic inference corroborates the initial identification performed by morphologic characteristics and specific PCR detection.

This is the first report of *R. oryzae-sativae* and *S. hydrophilum* associated with the sheath blight

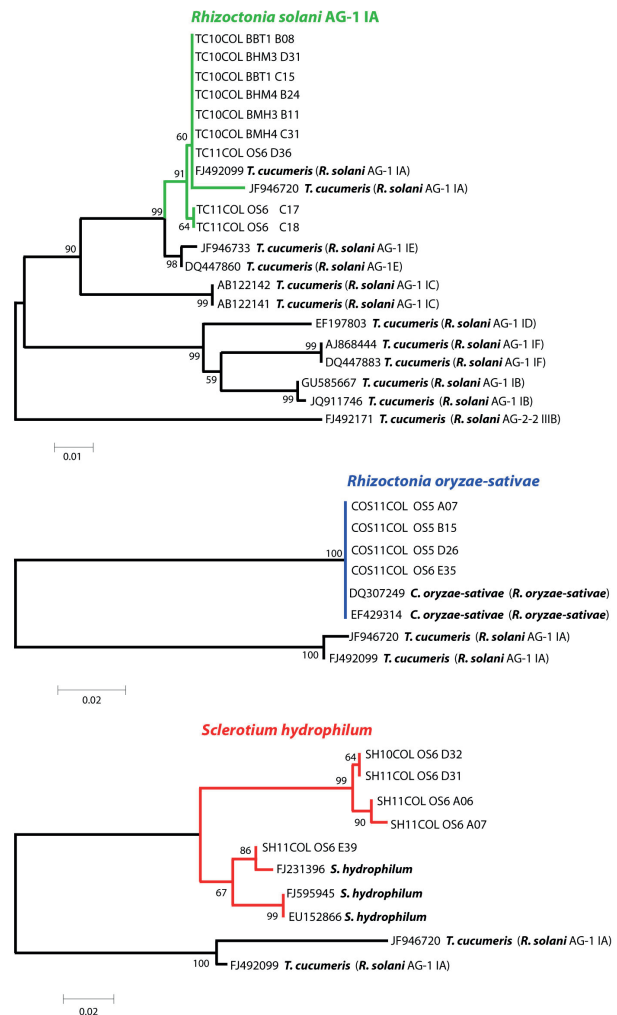


Figure 2. Phylogenetic trees, based on neighbor-joining distance, illustrating the relation among ITS-5.8S haplotypes of *Rhizoctonia solani* AG-1 IA, *R. oryzae-sativae* and *Sclerotium hydrophilum*. The bars indicate genetic distance among clades. Supporting values of bootstrap above 50 % are by each branch. The bold case names are sequences downloaded from NCBI/Genbank, to serve as control, of known species and anastomosis group, to aid classification.

and spot disease complexes of rice in Colombia. The predominance of *R. oryzae-sativae* in the two rice sampling areas in the Colombian Llanos (71 % of the population sampling) is particularly important for disease management, since *R. solani* AG-1 IA was considered the predominant pathogen in rice fields (Pabón Guerrero 1994). In South and North America, several species of the *Rhizoctonia* genus, such as *R. solani* AG-1 IA, *R. circinata* var. *oryzae*, *R. circinata* var. *zetae*, *R. circinata* var. *circinata*, *R. oryzae-sativae* and *Sclerotium* (*S. hydrophilum* and *S. oryzae*), are associated with the rice sheath blight and spot disease complexes in Argentina (Gutiérrez 2007), Brazil (Costa-Souza et al. 2007), Chile (Madariaga et al. 1999), USA (Lee & Rush 1983, Sayler & Yang 2007) and Venezuela (Cedeño et al. 1996).

Specifically, *R. solani* AG-1 IA is associated with sheath blight, *R. circinata* var. *oryzae* with sheath spot, *R. oryzae-sativae* with aggregated sheath spot, *S. hydrophilum* with sheath spot and *S. oryzae* with sheath rot (Gutiérrez 2007). The overlapping of similar symptoms reduces assertive diagnosis for these rice sheath diseases (Cedeño et al. 1996, Johanson et al. 1998). As diagnosis is a highly relevant step for establishing proper management strategies, especially with varietal resistance, simple methods are recommended, such as morphological characterization, to identify the associated pathogens.

The cross-pathogenicity assay in rice (cv. 'Fedearroz') showed that *R. oryzae-sativae* and *S. hydrophilum* are capable of inducing symptoms, however, with lower intensity than *R. solani* AG-1 IA isolates (Figure 3). On the other hand, *R. oryzae-sativae* and *S. hydrophilum* isolates practically do not induce symptoms in *U. brizantha* (cv. 'Toledo') (Figure 3).

R. solani AG-1 IA isolates obtained from *Urochloa* spp. populations were more aggressive to *U. brizantha* (cv. 'Toledo') than isolates obtained from rice (Figure 3). In rice, however, isolates from *Urochloa* spp. were similarly aggressive, when compared to rice isolates of *R. solani* AG-1 IA.

These findings support the hypothesis that *R. solani* AG-1 IA isolates infecting *Urochloa* spp. most likely originated from a population that originally infected rice via host shift (Chavarro Mesa et al. 2015). Similar examples are known to have occurred, such as host jump (from rice to soybean) or host shift (from rice to maize) of *R. solani* AG-1

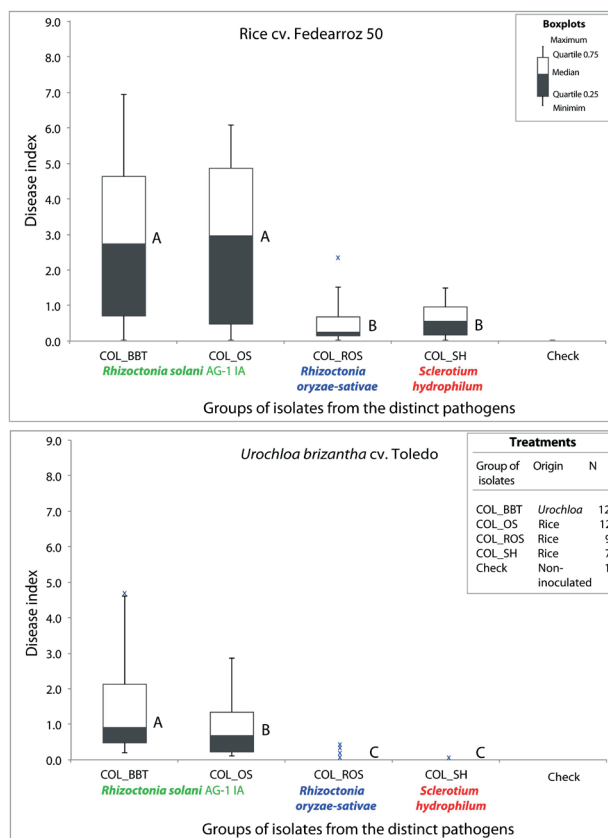


Figure 3. Aggressiveness of *Rhizoctonia solani* AG-1 IA, *R. oryzae-sativae* and *Sclerotium hydrophilum* groups of isolates from the Colombian Llanos to *Urochloa* and rice. Means followed by the same letter are not significantly different by contrast of means ($p \leq 0.01$).

IA populations in the southern United States (Assis et al. 2008) and Venezuela (González-Vera et al. 2010).

A recent study indicated that *R. solani* AG-1 IA isolates from *Urochloa* (syn. *Brachiaria*) were also pathogenic to cowpea and soybean (Chavarro Mesa et al. 2015). These results provide evidence that the *R. solani* AG-1 IA populations adapted to *Urochloa* spp. most likely are not genetically structured, and therefore keep a wide host range, extending also to the *Fabaceae* family.

CONCLUSIONS

1. *Rhizoctonia solani* AG-1 IA is the predominant pathogen associated with foliar blight in *U. brizantha* cv. 'Toledo' and hybrid *Urochloa* cv. 'Mulato', in the eastern Colombian Llanos.
2. *Urochloa* infecting *R. solani* AG-1 IA showed the highest disease intensity in *Urochloa* spp.

3. Isolates of *R. solani* AG-1 IA from rice or *Urochloa* are cross pathogenic in both plant species.
4. *R. oryzae-sativae* and *S. hydrophilum* were detected only in rice, but *R. oryzae-sativae* could produce very light symptoms in *Urochloa*.

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