RESEARCH ARTICLE

Pathogenicity of *Rhizoctonia solani* AG 1-IA on major weeds prevalent in rice and maize ecosystem in Meghalaya

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ABSTRACT: *Rhizoctonia solani* Kühn (Teleomorph: *Thanatephorus cucumeris*) is an economically important soil borne basidiomycetous pathogen of worldwide distribution. It causes sheath blight disease of rice and banded leaf and sheath blight (BLSB) disease in maize. Twenty three lowland rice weeds and thirty one upland (maize) weeds were collected and used. Rice isolate (SRS) was pathogenic on all the lowland rice and upland weeds. Maize isolate (RSM2) was not pathogenic on *Cyperus difformis*, *C. haspans*, *C. odoratus*, *Sagittaria sagittifolia*, *Celosia argentea*, *Commelina diffusa* and *Floscopa scandens*. Minimum days for sclerotia formation was 3 days on *C. bengalensis*, maximum sclerotia production was observed on *Echinochloa crusgalli* (14 nos.) with the isolate SRS. Minimum days for sclerotia formation was 2 days after inoculation on *Euphorbia hirta*, *Fimbristylis dichotoma*, *Rotala indica* and *Scirpus juncooides*. Maximum sclerotia production was observed on *Alternanthera philoxeroides* (11 nos.) with the isolate RSM2. Area under disease progress curve was calculated and mean separation was done using REGWQ test (Ryan/Elinot/Gabriel/Welsch procedure). The weeds *Ageratum houstonianum* (10.2), *A. sessilis* (14), *A. philoxeroides* (25.3), *Imperata cylindrica* (14), *Paspalum distichum* (17.8) and *Ambrosia artemisiifolia* (23.3) were susceptible whereas *Dactyloctenium aegyptium* (115.6), *E. colona* (115.6) and *Bidens pilosa* (111.9) were highly susceptible to the isolate SRS. The weed *A. houstonianum* (43.7) was susceptible and *R. indica* (267.4) and *E. hirta* (266.2) were highly susceptible to the isolate RSM2.

Key words: AG 1-IA, anastomosis group, pathogenicity test, *Rhizoctonia solani*

The sheath blight of rice caused by *Rhizoctonia solani* is currently ranked second after blast disease (Prasad *et al.*, 2010). Banded leaf and sheath blight of maize caused by *R. solani* is one of the most widespread and destructive diseases found in most parts of the world (Rani *et al.*, 2013). The pathogen *R. solani* is a non-obligate, necrotrophic, phytopathogenic basidiomycetous fungus causing severe economic damage to a wide variety of plant species. It has limited movement due to lack of spores and it survives in unfavourable conditions in form of sclerotia or dormant mycelia (Sumner, 1996). Sclerotia can survive for two years in the soil, they may float in permanent flooded conditions and move to nearby fields or within the field through continuous flood irrigation and then they get attached to the plant causing sheath blight disease (Brooks, 2007). *R. solani* is composed of different anastomosis groups (AGs). Hyphal anastomosis is the traditional method of differentiating and grouping strains. Anastomosis is the fusion of two compatible vegetative hyphae. Based on anastomosis reactions like hyphal fusion, morphology, pathogenicity and physiology, 14 AGs have been identified till date (AG1 to AG13 and AG-BI) (Carling, 1996; Gonzalez *et al.*, 2006; Wang *et al.*, 2015).

The pathogen has worldwide distribution and is known to attack at least 188 species of higher plants,

including crops, vegetables, ornamentals, forest trees and turfgrasses (Lakshman and Amaradasa, 2014). In different agro-climatic regions of India, several weeds and cultivated plant species are known to act as alternate and collateral hosts of *R. solani* (Singh *et al.*, 2012).

Present study was taken up to understand the pathogenicity of AG1-IA on weed hosts, which in turn would make possible to evolve novel strategies to control sheath blight disease on rice, maize and several other crops of agricultural importance.

MATERIALS AND METHODS

Samples infected with *R. solani* which exhibited sheath blight and banded leaf and sheath blight symptoms on rice and maize, respectively were collected and isolation was done following the method described by Mahendra *et al.* (2016) with minor modifications (antibiotic Chloramphenicol @ 50 mg/l was suspended in 10 ml of 95% alcohol then it was mixed with one liter of media before sterilization). Pure cultures were obtained by cutting hyphal tips from the periphery of actively grown 3-day old cultures and were transferred to PDA slants. Thereafter, isolates were designated as SRS and FRS, both obtained from rice, RSM1 and RSM2, both obtained from maize for use in further studies.

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Initial pathogenicity test of rice and maize isolates

Pathogenicity was confirmed by inoculation of rice isolates SRS and FRS and maize isolates RSM1 and RSM2 on maize variety (DA-61A) and on rice variety (HR12) using the method described by Mahendra *et al.* (2016).

Molecular characterization of *R. solani* isolates by using specific primers

The DNA extraction and PCR was done following the methods (with minor modifications) mentioned by Mahendra *et al.* (2016). Presence of specific bands using specific primers was used as criteria for identification of anastomosis groups and subgroups of *R. solani* (Matsumoto, 2002; Saylor and Yang, 2007). One isolate (SRS) was sequenced after obtaining the product using universal primers for ITS region (ITS 5 and 4) (White *et al.*, 1990). The sequence was deposited in GenBank. Using BLASTn similarity search was performed. Sequences of other AGs were downloaded from NCBI and included in the phylogenetic analysis. The "Maximum Parsimony (MP)" analysis was done using the Subtree pruning and regrafting algorithm in which the initial trees were obtained with the random addition of sequences (Tamura *et al.*, 2013). Nodal support was calculated using one thousand bootstrap replicates (Felsenstein, 1985) and the ITS sequence of *Athelia rolfsii* (AY684917) was used as root.

Pathogenicity of *R. solani* isolates on major weeds of lowland rice

Major lowland weeds prevalent in and around paddy field, water channel and irrigation ponds were collected. For pathogenicity test, detached leaves of weeds were used. Three days old culture of SRS and RSM2 isolates of *R. solani* were used for inoculation, incubated at 27±1°C and examined regularly for appearance of symptoms and production of sclerotia.

Pathogenicity test of *R. solani* on major weeds of maize

Major upland weeds prevalent in and around the maize field and upland paddy field were collected. The pathogenicity test was carried out using isolates SRS and RSM2 on detached leaves of weeds. After inoculation symptom appearance was checked regularly and per cent infected area was recorded on all replicates. Production of sclerotia was also observed and recorded after inoculation.

Pathogenicity test on different crops

Eight different crops namely ginger (*Zingiber officinale*), groundnut (*Arachis hypogaea*), turmeric (*Curcuma longa*), soyabean (*Glycine max*), cowpea (*Vigna unguiculata*), red gram (*Cajanus cajan*), wild ginger (*C. angustifolia*) and mesta (*Hibiscus sabdariffa*) commonly cultivated in vegetable garden were collected. The

pathogenicity test was carried out on apparently healthy detached leaves of crops using both the isolates SRS and RSM2. Symptom occurrence was checked regularly after inoculation and per cent infected area was recorded.

Statistical analysis

Statistical analysis was done using SPSS 20.0 software. The area under disease progress curve (AUDPC) was calculated using the following formula.

$$AUDPC = \sum_{i=1}^k \frac{1}{2} (S_i + S_{i-1}) (t_{i+1} - t_i)$$

RESULTS AND DISCUSSION

Molecular characterization

Four isolates were identified by PCR amplification using specific primers. All the isolates belonged to AG 1-IA. Based on amplification with specific primers and band sizes the isolates were identified as AG 1-IA (~265 bp and ~140 bp). The isolates RSM2, RSM1 and SRS gave a band size of 265 bp. In case of isolate FRS, the band size was ~140 bp since second primer set for identification of AG 1-IA was used. The ITS region of one isolate (SRS) was obtained using universal primers (ITS 5 and 4) and the sequence has been deposited in GenBank (KX132899). Similarity searches using Blastn showed 100% similarity with AG 1-IA sequences. Phylogenetic analysis using maximum parsimony also grouped it with AG 1-IA with 100% bootstrap support (Fig. 1).

Matsumoto (2002) and Saylor and Yang (2007), have designed specifically primed PCR for direct detection of AG 1 and AG 2 subgroups and using these primers, the four isolates SRS, FRS, RSM1 and RSM2 were identified as AG 1 IA. Baiswar *et al.* (2012 and 2014) have also identified AG 1-IA on rice and maize, and AG 1 IB on soybean, marigold using the primers developed by Matsumoto (2002) and Saylor and Yang (2007).

Initial pathogenicity test with rice and maize isolates

Prominent symptoms on the inoculated leaf sheaths of rice were observed after 4 days of inoculation on both the replicates. Whereas, in case of maize, symptoms on the inoculated leaves were observed after 2 days of inoculation on both the replicates. The rice isolate SRS was more virulent than FRS and maize isolate RSM2 was more virulent than RSM1. So, SRS and RSM2 were used for further pathogenicity test on lowland rice and maize weeds.

Pathogenicity test of *R. solani* isolates on major weeds of lowland rice

The isolate SRS was pathogenic on all weeds as shown in the Table 1. The isolate RSM2 was found to be pathogenic on all weeds except *C. difformis*, *C. haspans*, *C. odoratus* and *S. sagittifolia*.

Fig. 1. Maximum parsimony tree obtained using subtree pruning and regrafting algorithm

Although both the isolates belonged to AG 1-IA, this difference in pathogenicity may be due to high intra-group variability among the subgroup according to Susheela and Reddy (2013). Moni *et al.* (2016) observed significant genetic variation within the isolates of *R. solani* collected from similar agro-ecological regions of Bangladesh. This study revealed that *R. solani* has the ability to infect wide range of weeds and these weeds act as alternate host of *R. solani*. Similar findings have also been reported earlier by Acharya and Sengupta (1998), Sivalingam *et al.* (2006) and Lenka *et al.* (2014). They found weeds like *Amaranthus viridis*, *Commelina benghalensis*, *Cynodon dactylon*, *Dactyloctenium aegyptium*, *Digitaria ciliaris*, *Echinochloa colona*, *Euphorbia hirta*, *Fimbristylis miliacea* etc. as alternate host of *R. solani*. Mareeswari *et al.* (2004) have also reported that the weeds like *C.*

dactylon, *E. crusgalli*, *Marsilea quadrifoliata* and *Typha angustata* are highly susceptible to infection by *R. solani*.

Pathogenicity test of *R. solani* on major weeds of maize

The isolate SRS was pathogenic on all the weeds as shown in Table 2. The isolate RSM2 was pathogenic on all weeds except *C. argentea*, *C. diffusa* and *F. miliacea* (Fig. 2).

Khandaker *et al.* (2008) also tested pathogenicity of two *R. solani* isolates on 33 different hosts and they observed differences in the degree of pathogenicity among the hosts.

Table 1. Pathogenicity test on weeds of lowland rice

Scientific name	Pathogenicity test with rice isolates (SRS)	Pathogenicity test with maize isolates (RSM2)
<i>Alternanthera philoxeroides</i>	+	+
<i>Brachiaria mutica</i>	+	+
<i>Bulbostylis densa</i>	+	+
<i>Commelina benghalensis</i>	+	+
<i>Cynodon dactylon</i>	+	+
<i>C. difformis</i>	+	-
<i>C. haspans</i>	+	-
<i>C. iria</i>	+	+
<i>C. odoratus</i>	+	-
<i>C. pilosus</i>	+	+
<i>Echinochloa colona</i>	+	+
<i>E. crusgalli</i>	+	+
<i>Eleocharis congesta</i>	+	+
<i>Emilia sonchifolia</i>	+	+
<i>Eragrostis unioides</i>	+	+
<i>Euphorbia hirta</i>	+	+
<i>Fimbristylis dichotoma</i>	+	+
<i>F. miliacea</i>	+	+
<i>Monochoria vaginalis</i>	+	+
<i>Paspalum dilatatum</i>	+	+
<i>Rotala indica</i>	+	+
<i>Sagittaria sagittifolia</i>	+	-
<i>Scirpus juncooides</i>	+	+

+ pathogenic, - non pathogenic

The findings of the present study confirmed that the pathogen could reside in the weeds of maize and these infected weeds could act as a source of inoculum. So, it is recommended to remove the weed hosts to reduce the inoculum potential. Gokulapalan and Nair (1983) also found pathogen *R. solani* residing in weed hosts viz. *Alternanthera philoxeroides*, *C. iria*, *E. colona* and *E. crusgalli* and revealed the importance of weed control which would reduce plant competition and also increase the yield.

Fig. 2. Pathogenicity test with isolate RSM2 on weed *Digitaria ciliaris***Table 2.** Pathogenicity test on weeds of maize

Scientific name	Pathogenicity test with rice isolates (SRS)	Pathogenicity test with maize isolates (RSM2)
<i>Ageratum houstonianum</i>	+	+
<i>Alternanthera sessilis</i>	+	+
<i>Amaranthus spinosus</i>	+	+
<i>Ambrosia artemisiifolia</i>	+	+
<i>Ammannia baccifera</i>	+	+
<i>Bidens pilosa</i>	+	+
<i>Celosia argentea</i>	+	-
<i>Commelina benghalensis</i>	+	+
<i>C. diffusa</i>	+	-
<i>Crassocephalum crepidioides</i>	+	+
<i>Cuphea balsamona</i>	+	+
<i>Cynodon dactylon</i>	+	+
<i>Cyperus iria</i>	+	+
<i>Cyrtococcum accrescens</i>	+	+
<i>Dactyloctenium aegyptium</i>	+	+
<i>Digitaria adscendens</i>	+	+
<i>D. ciliaris</i>	+	+
<i>Echinochloa colona</i>	+	+
<i>E. crusgalli</i>	+	+
<i>Eleusine indica</i>	+	+
<i>Euphorbia hirta</i>	+	+
<i>Fimbristylis dichotoma</i>	+	+
<i>F. miliacea</i>	+	+
<i>Floscopa scandens</i>	+	-
<i>Galinsoga parviflora</i>	+	+
<i>Imperata cylindrica</i>	+	+
<i>Ischaemum rugosum</i>	+	+
<i>Opismenus composites</i>	+	+
<i>Paspalum distichum</i>	+	+
<i>Setaria glauca</i>	+	+
<i>Spilanthes acmella</i>	+	+

+ pathogenic, - non pathogenic

Sclerotial count and days required for sclerotia formation on lowland rice and maize weeds with rice isolate (SRS)

Sclerotial count was recorded after inoculation with the SRS isolate. Number of sclerotia production ranged from 1 to 14 as shown in the Fig 3. The isolate SRS showed great variation in time taken for sclerotia formation, which ranges from 3 to 7 days after inoculation as shown in Fig 3.

Debbarma and Dutta (2015) also observed great variation in time taken for sclerotia formation. It ranged from 9 to 18 days with *R. solani* isolates obtained from rice, maize, cowpea, greengram and pepper. Singh *et al.* (2015) also observed variation in no. of sclerotia produced by different isolates of *R. solani* obtained from maize, rice, potato, mungbean and urd bean. Similarly, Singh *et al.* (2015) also reported that *R. solani* isolates showed great variation in the time taken for sclerotia

Fig. 3. Sclerotial count and days required for sclerotia formation on weeds with rice isolate (SRS)

formation which ranged from 3 to 6 days. Previous finding indicates that in tropical environment sheath blight pathogen *R. solani* survives through sclerotia from one crop season to another and mycelia through weed hosts and in plant debris (Kobayashi *et al.*, 1997). So, it is necessary to remove the weed hosts.

Sclerotial count and days required for sclerotia formation on lowland rice and maize weeds with maize isolate (RSM2)

Sclerotial count was recorded after inoculation with isolate RSM2. Number of sclerotia production ranged from 1 to 11 as shown in Fig 4. The isolate RSM2 showed variation in time taken for sclerotia formation, which ranged from 2 to 6 days after inoculation.

Variation in number of sclerotia production has been also documented by Singh *et al.* (2015) using different isolates of *R. solani* obtained from maize, rice and potato. Ganeshamoorthi and Dubey (2015) also reported a high variation in number of sclerotia formation, ranging from 1 to 9 with different isolates of chickpea, mungbean and urdbean. Similarly, Meena *et al.* (2001) also reported that the time taken for sclerotia formation ranged from 3 to 6 days. Variation in time taken for sclerotia formation has also been reported by Singh *et al.* (2015) using different isolates of *R. solani* obtained from maize, rice and potato.

Pathogenicity test on different crops

The isolates SRS and RSM2 were used for pathogenicity test on eight different plant species. The isolate SRS was pathogenic on all the plant species (Fig. 5). The isolate

Fig. 4. Sclerotial count and days required for sclerotia formation on weeds with maize isolate (RSM2)

Fig. 5. Pathogenicity test with isolate SRS on Groundnut

RSM2 was pathogenic on *V. unguiculata*, *H. sabdariffa*, *C. cajan* and *C. angustifolia* but it was not able to infect four crops viz., *Z. officinale*, *A. hypogaea*, *G. max* and *C. longa*.

Many other research workers like Acharya and Sengupta (1998), Gokulapalan and Nair (1983), Meena and Muthusamy (1998) and Nelson *et al.* (1996) have also reported groundnut, sugarbeet, dry bean, mustard, sunflower and flux seedlings etc. as collateral hosts of the sheath blight pathogen.

Susceptibility analysis using AUDPC of lowland rice and maize weeds with rice isolate (SRS)

The results revealed that weeds namely, *Ageratum houstonianum* (10.2), *A. sessilis* (14), *A. philoxeroides* (25.3), *Imperata cylindrica* (14), *Paspalum distichum*

Table 3. AUDPC of weeds inoculated with rice isolate (SRS)

Scientific name	AUDPC*
<i>Ageratum houstonianum</i>	10.2 ^a
<i>Alternanthera sessilis</i>	14.0 ^a
<i>Amaranthus spinosus</i>	55.2 ^{bc}
<i>Ambrosia artemisiifolia</i>	23.3 ^a
<i>Ammannia baccifera</i>	44.1 ^b
<i>Bidens pilosa</i>	111.9 ^h
<i>Commelina benghalensis</i>	80.2 ^{ef}
<i>C. diffusa</i>	76.1 ^{de}
<i>Dactyloctenium aegyptium</i>	115.6 ^h
<i>Digitaria adscendens</i>	93.0 ^g
<i>Echinochloa colona</i>	115.6 ^h
<i>Fimbristylis dichotoma</i>	75.7 ^{de}
<i>Imperata cylindrica</i>	14.0 ^a
<i>Oplismenus compositus</i>	93.0 ^g
<i>Paspalum distichum</i>	17.8 ^a
<i>Setaria glauca</i>	93.0 ^g
<i>Alternanthera philoxeroides</i>	25.3 ^a
<i>Brachiaria mutica</i>	105.9 ^{gh}
<i>Bulbostylis densa</i>	93.0 ^g
<i>Cyperus odoratus</i>	102.7 ^{gh}
<i>Eragrostis unioides</i>	64.7 ^{cd}
<i>Scirpus juncooides</i>	59.8 ^c

*Arcsine transformed values

Means within a column with same letters are not significantly different according to REGWQ test ($p=0.05$) (Ryan/Einot/Gabriel/Welsch procedure)

(17.8) and *Ambrosia artemisiifolia* (23.3) which are statistically at par were least susceptible whereas *D. aegyptium* (115.6), *E. colona* (115.6) and *Bidens pilosa* (111.9) were at par and highly susceptible to the isolate SRS (Table 3).

Susceptibility analysis using AUDPC of lowland rice and maize weeds with maize isolate (RSM2)

The results revealed that the weed *A. houstonianum* (43.7) was least susceptible whereas weeds like *Rotala indica* (267.4) and *E. hirta* (266.2) were highly susceptible and statistically at par, using AUDPC criteria (Table 4).

Adhipathi *et al.* (2013) have used this criteria for identifying susceptibility against sheath blight of rice. The AUDPC is considered important since it provides an estimate for disease progress overtime rather than just the final severity.

It can be concluded from the present study that most of the upland and lowland weeds are susceptible to *R. solani* AG 1-IA but few are highly susceptible and these highly susceptible weeds should not be used as mulching material. Also, removal of these weeds will ultimately help in managing the sheath blight of rice and banded leaf and sheath blight of maize.

Table 4. AUDPC of weeds inoculated with maize isolate (RSM2)

Scientific name	AUDPC*
<i>Ageratum houstonianum</i>	43.7 ^a
<i>Bidens pilosa</i>	146.4 ^{fg}
<i>Commelina benghalensis</i>	171.9 ^g
<i>Cynodon dactylon</i>	115.6 ^{de}
<i>Cyperus iria</i>	71.3 ^{abc}
<i>Dactyloctenium aegyptium</i>	93.0 ^{cd}
<i>Digitaria adscendens</i>	108.1 ^d
<i>Echinochloa colona</i>	155.8 ^g
<i>E. crusgalli</i>	151.8 ^g
<i>Eleusine indica</i>	142.9 ^{efg}
<i>Euphorbia hirta</i>	266.2 ⁱ
<i>Galinsoga parviflora</i>	98.3 ^{cd}
<i>Imperata cylindrica</i>	76.6 ^{bc}
<i>Paspalum distichum</i>	99.0 ^{cd}
<i>Ammannia baccifera</i>	160.8 ^g
<i>Alternanthera philoxeroides</i>	119.3 ^{def}
<i>Bulbostylis densa</i>	93.0 ^{cd}
<i>Cyperus pilosus</i>	205.2 ^h
<i>Emilia sonchifolia</i>	108.1 ^d
<i>Eragrostis unioides</i>	120.9 ^{def}
<i>Paspalum dilatatum</i>	49.9 ^{ab}
<i>Rotala indica</i>	267.4 ⁱ
<i>Scirpus juncooides</i>	164.7 ^g

*Arcsine transformed values

Means within a column with same letters are not significantly different according to REGWQ test ($p=0.05$) (Ryan/Einot/Gabriel/Welsch procedure)

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