Indian Journal of Weed Science **50**(1): 72–76, 2018

Print ISSN 0253-8040 Online ISSN 0974-8164

Pathogenicity of *Rhizoctonia solani* AG 1-IB on common weeds in Meghalaya

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Article information

DOI: 10.5958/0974-8164.2018.00016.3

Type of article: Research article

Received : 27 October, 2017
Revised : 2 March 2018
Accepted : 5 March 2018

Key words
AG 1-IB
Rhizoctonia solani
Pathogenicity

ABSTRACT

Rhizoctonia solani Kuhn is a soil borne fungal plant pathogen, infecting several crops. Many weeds act as collateral hosts of this pathogen and help in spreading this. Pathogenicity of *R. solani* AG 1-IB isolate was tested on 47 common weeds of Meghalaya. Incubation period on all the common weeds was 2-3 days except 6 days on *Cyperus iria* with *R. solani* AG 1-IB isolate. Minimum days for sclerotia formation was 4 days on *Crassocephalum crepidioides, Galinsoga parviflora* and *Tridax procembens*. Maximum sclerotia production (16 nos.) was observed on *Lantana camara*. The weed *Emilia sonchifolia* was most susceptible to isolate *R. solani* AG 1-IB based on area under disease progress curve criteria. Highly susceptible weeds identified in this study should be avoided for mulching purpose since this will increase the inoculum load of this pathogen

INTRODUCTION

The pathogen *Rhizoctonia solani* Kuhn is a soil borne fungal plant pathogen that attacks various cereals, vegetables, legumes, forest trees, ornamentals and turf grasses, causing significant losses (Lakshman *et al.* 2016). It causes various plant diseases like collar rot, root rot, damping off, sheath blight, banded leaf and sheath blight, stem canker, web blight, and wire stem in different plant species throughout the world (Debbarma and Dutta 2015). The pathogen forms sclerotia under unfavourable conditions and it survives in the soil for around two years. The fungus *R. solani* also survives as mycelium by colonizing soil organic matter as a saprophyte. Sclerotia or mycelium present in soil and on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of crops. The fungus *R. solani* has been classified into different anastamosis groups (AGs) on the basis of hyphal anastomosis reactions. These groups are considered to be genetically isolated (Carling 1996). Till date, *R. solani* contains 13 AG groups and AG IB (Carling *et al.* 2002).

Several weeds and cultivated plant species are known to act as alternate and collateral hosts of *R. solani* in different agro-climatic regions of India (Chahal *et al.* 2003 and Saveinai *et al.* 2017). Information related to pathogencity of *R. solani* on different weeds is scarce. Hence, this study was carried out to explore to find out the susceptible weed species to plan a better management of crops and weeds.

MATERIALS AND METHODS

Experiments related to "Pathogenicity of *R. solani* AG 1-IB on common weeds in Meghalaya" were conducted at ICAR Research Complex for NEH Region, Umiam, Meghalaya during 2016-2017. Infected soybean samples exhibiting web blight symptoms were collected from experimental plots of ICAR, Umiam and isolation was done following the method as described in Saveinai (2016) with minor modifications. Isolates (pure cultures) were designated as PPJ1, PPJ2, PPJ3, PPJ4 and PPJ5. Microscopic observations were performed on soybean isolates for confirmation of the fungus as *Rhizoctonia* spp. based on hyphal characters using Olympus BX 53 microscope.

PCR was used for amplification of extracted DNA with specific primers for AG 1-IA and IB (Matsumoto 2002 and Sayler and Yang 2007). Both positive and negative controls were also used.The DNA extraction, PCR (with minor modifications), gel documentation and identification was done following the procedure described by Mahendra *et al.* (2016).One isolate (PPJ3) was also amplified and

sequenced by using universal primers for ITS region (ITS 5 and 4) (White *et al*. 1990) and the sequence was deposited in Genbank. Similarity search was also performed using BLASTn. Sequences of other AGs were downloaded from NCBI and included in the phylogenetic analysis using Maximum parsimony method in MEGA 6 (using Subtree pruning and regrafting algorithm) (Tamura *et al*. 2013). Nodal support was calculated using one thousand bootstrap replicates (Felsenstein 1985).

Initial pathogenicity was done by inoculation of *R. solani* AG 1-IB isolates PPJ1, PPJ2, PPJ3, PPJ4 and PPJ5 on soybean by using detached leaf method (Rahayu 2014). Same method was used for testing pathogenicity of PPJ3 isolate on common weeds. The area under disease progress curve (AUDPC) was calculated and analysis was done as mentioned in Saveinai *et al*. (2017).

RESULTS AND DISCUSSION

Molecular identification of *Rhizoctonia solani* AG 1-IB

Five isolates collected were identified by using specific primers. Based on amplification with specific primers and band size (~300 bp) the isolates were identified as AG 1-IB. The ITS region of one isolate (PPJ3) was obtained using universal primers and the resulting sequence has been deposited in Genbank (KY172991). Similarity searches using BLASTn showed 99% similarity with AG 1-IB sequences (KF907717, JQ692292). Phylogenetic analysis using maximum parsimony also clustered the isolate with AG 1-IB with 81% bootstrap support (**Figure 1**). Baiswar *et al*. (2012) have also identified AG 1-IB on soybean and marigold using the primers developed by Matsumoto (2002) and Saylor and Yang (2007).

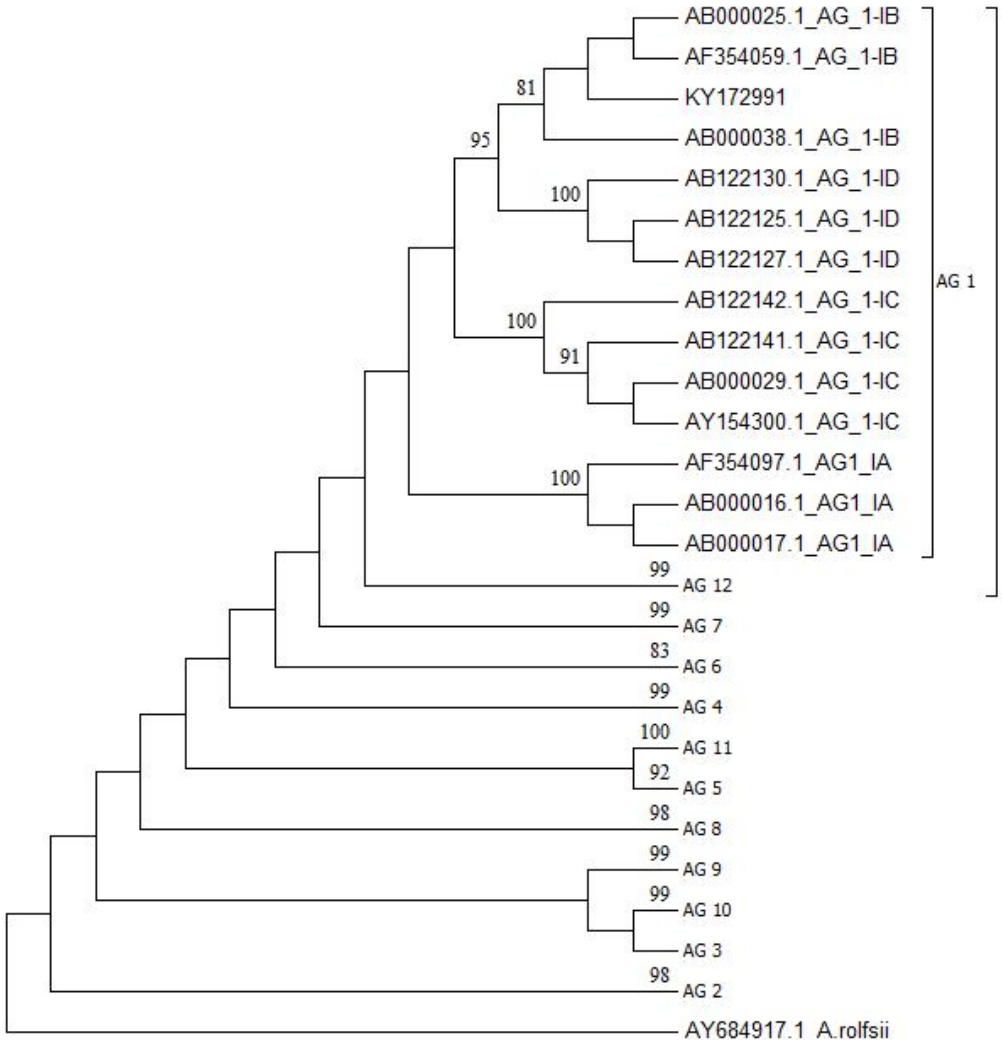

Figure 1. Phylogenetic analysis of *Rhizoctonia solani* isolate PPJ3. The ITS sequence of *Athelia rolfsii* (AY684917) was used as root. All the nodes except AG 1 have been collapsed to improve the clarity. The sequence IDs of collapsed AGs are mentioned in Saveinai *et al*. (2017)

Phylogenetic approach utilizing ITS region has also been used by several workers for accurate identification (Baiswar 2012; Mahendra *et al*. 2016 and Savenai *et al*. 2017).

Pathogenicity of *Rhizoctonia solani* AG 1-IB on common weeds

Initial pathogenicity was done by inoculation of *R. solani* AG 1-IB isolates PPJ1, PPJ2, PPJ3, PPJ4 and PPJ5 on soybean by using detached leaf method. All the isolates were found to be virulent and isolate PPJ3 was used for further pathogenicity test on common weeds.

Rhizoctonia solani AG 1-IB isolate PPJ3 was pathogenic on all the 47 common weeds (**Table 1**). Kannaiyan and Prasad (1979) have also reported that *R. solani* occured on 21 weed hosts and it was severe on weeds like *Eriochloa procera, Andropogon asper, Cynodon dactylon* and *Ischaemum indicum*. Saveinai *et al*. (2017) did similar kind of work on pathogeniticity of *R. solani* AG 1-IA on weeds and results showed that isolate SRS (isolated from rice) was pathogenic on all the weeds and isolate RSM2 (isolated from maize) was found to be pathogenic on all weeds except *Cyperus difformis, C. haspan*s, *C. odoratus, S. sagittifolia, Celosia argentea, Commelina diffusa* and *F. miliacea.* In our findings, *R. solani* AG 1-IB (isolated from soybean) was pathogenic on all the weeds. This clearly demonstrates that different AG subgroups and even different isolates of same AG subgroup differ in there pathogenicity against different weeds.

Incubation period, minimum days taken for sclerotia formation and maximum number of sclerotia on common weeds with *Rhizoctonia solani* AG 1-IB isolate

Incubation period on all the common weeds varied from 2-6 days (**Table 1**) *i.e*. two days on *Ageratum houstonianum, Alternanthera sessilis, Amaranthus viridis, Ambrosia artemisiifolia, Arundinella mutica, Bidens pilosa, Borreria latifolia, , Celosia argentea, Commelina benghalensis, Crassocephalum crepidioides, Crotalaria striata, Cuphea balsamona, Cynodon dactylon, Cyperus rotundus, Digitaria adscendens, Echinochloa colona, Eleucine indica, Emilia sonchifolia, Erigeron bonariensis, Eupatorium adenophorum, Euphorbia hirta, Fagopyrum esculantum, Galinsoga parviflora, Ipomoea* sp., *I. indica, Lantana camara, Mikania micrantha, Rotala indica, Sida carpinifolia, Sida rhombifolia, Spermacoce latifolia, Spilanthes paniculata, Stachytarpheta indica, Tridax procumbens* and *Urena lobata* three days on *A. philoxeroides, A. bengalensis, Cyrtoccum accrescens, E. crusgalli, E. unioloides, Fimbristylis miliacea, P. dilatatum, P. scrobiculatum, Scirpus juncoides* and *Setaria glauca.* Incubation period of four days on *I. rugosum* and six days was observed on *C. iria* with *R. solani* AG 1-IB isolate.

Table 1. Pathogenicity, incubation period, minimum days taken for Sclerotia formation and maximum number of sclerotia on common weeds with *Rhizoctonia solani* AG 1-IB isolate

Weed	Pathog-enicity	Incuba-tion period (days)	Sclerotia formation (minumum days taken)	Maximum no. of sclerotia
Ageratum houstonianum	+	2	11	4
Alternanthera philoxeroides	+	3	-	-
Alternanthera sessilis	+	2	15	9
Amaranthus viridis	+	2	8	2
Ambrosia artemisiifolia	+	2	8	7
Arundinella bengalensis	+	3	12	3
Arundinella mutica	+	2	12	4
Bidens pilosa	+	2	5	8
Borreria latifolia	+	2	5	13
Celosia argentea	+	2	8	7
Commelina benghalensis	+	2	11	2
Crassocephalum crepidioides	+	2	4	12
Crotalaria striata	+	2	7	4
Cuphea balsamona	+	2	7	3
Cynodon dactylon	+	2	10	3
Cyperus iria	+	6	10	2
Cyperus rotundus	+	2	10	3
Cyrtoccum accrescens	+	3	12	2
Digitaria adscendens	+	2	9	9
Echinochloa colona	+	2	10	2
Echinochloa crusgalli	+	3	10	8
Eleucine indica	+	2	7	6
Emilia sonchifolia	+	2	8	4
Eragrostis unioloides	+	3	12	3
Erigeron bonariensis	+	2	12	3
Eupatorium adenophorum	+	2	12	3
Euphorbia hirta	+	2	5	5
Fagopyrum esculantum	+	2	8	3
Fimbristylis miliacea	+	3	12	7
Galinsoga parviflora	+	2	4	6
Ipomoea indica	+	2	-	-
Ipomoea sp.	+	2	12	7
Ischaemum rugosum	+	4	9	4
Lantana camara	+	2	9	16
Mikania micrantha	+	2	8	8
Paspalum dilatatum	+	3	9	7
Paspalum scrobiculatum	+	3	10	5
Rotala indica	+	2	10	1
Scirpus juncoides	+	3	10	1
Setaria glauca	+	3	9	2
Sida carpinifolia	+	2	8	6
Sida rhombifolia	+	2	8	4
Spermacoce latifolia	+	2	7	4
Spilanthes paniculata	+	2	10	3
Stachytarpheta indica	+	2	-	-
Tridax procumbens	+	2	4	12
Urena lobata	+	2	8	6

Minimum days taken for sclerotia formation on common weeds with *R. solani* AG 1-IB isolate showed great variation, these ranged from 4 to 15 days after inoculation (**Table 1**. Minimum days taken for sclerotia formation was observed on weeds *C. crepidioides, G. parviflora* and *T. procumbens,* which took 4 days. Three weeds *B. pilosa, B. latifolia* and *E. hirta* took 5 days for sclerotia formation. Four weeds *C. striata, C. balsamona, E. indica* and *S. latifolia* took 7 days. Weeds, *A. viridis,A. artemisiifolia, C. argentea, E. sonchifolia, F. esculantum, M. micrantha, S. carpinifolia, S. rhombifolia* and *U. lobata* took 8 days. Five weeds *D. adscendens, I. rugosum, L. camara, P. dilatatum* and *S. glauca* took 9 days. Nine weeds took 10 days (*C. dactylon, C. iria, C. rotundus, E. colona, E. crusgalli, P. scrobiculatum, R. indica, S. juncoides* and *S. paniculata*). Two weeds *A. houstonianum* and *C. benghalensis* took 11 days. Weeds, *A. bengalensis, A. mutica, C. accrescens, E. unioloides, E. bonariensis, E. adenophorum, F. miliacea* and *Ipomoea* sp. took 12 days for sclerotia formation. Maximum days taken for sclerotia formation (15 days) was observed on weed *A. sessilis*. Rajput and Harlapur (2016) observed great diversity for time taken for initiation of sclerotia formation ranging from 8 to 15 days with *R. solani* isolates.

Number of sclerotia production using *R. solani* AG 1-IB isolate ranged from 1 to 16 (**Table 1**). Maximum sclerotia production (16 nos.) was observed on weed *L. camara*. Sclerotia were produced on almost all the weeds, *viz. B. latifolia* (13 nos.), *C. crepidioides* and *T. procumbens* (12 nos.), *A. sessilis* and *D. adscendens* (9 nos.), *B. pilosa, E. crusgalli* and *M. micrantha* (8 nos.), *A. artemisiifolia, C. argentea, F. miliacea, Ipomoea* sp. and *P. dilatatum* (7 nos.), *E. indica, G. parviflora, S. carpinifolia* and *U. lobata* (6 nos.), *E. hirta* and *P. scrobiculatum* (5 nos.), *A. houstonianum, A. mutica, C. striata, E. sonchifolia, I. rugosum, S. rhombifolia* and *S. latifolia* (4 nos.), *A. bengalensis, C. balsamona, C. dactylon, C. rotundus, E. unioloides, E. bonariensis, E. adenophorum, F. esculantum* and *S. paniculata* (3 nos.), *A. viridis, C. benghalensis, C. iria, C. accrescens, E. colona* and *S. glauca* (2 nos.). Minimum sclerotia production (1 no.) was observed on weeds *R. indica,* and *S. juncoides*. No sclerotia production was observed on weeds *A. philoxeroides, I. indica* and *S. indica*.

AUDPC (Area under the disease progress curve) used for susceptibility analysis of common weeds with *Rhizoctonia solani* AG 1-IB isolate

The weed *E. sonchifolia* (195.1) was most susceptible to isolate *R. solani* AG 1-IB followed by *C. argentea* (187.1), *T. procumbens* (180.0), *G. parviflora* (174.1) and *C. crepidioides* (172.7) were statistically at par (**Figure 2**), followed by *M. micrantha* (159.0), *A. viridis* (157.7), *S. rhombifolia* (148.2) and *A. artemisiifolia* (128.4). The weeds *S. carpinifolia* (118.5) and *R. indica* (113.4) were statistically at par followed by *U. lobata* (105.1). The AUDPC in case of weeds *C. striata, S. latifolia* and *B. pilosa* was 101.3, 100.8 and 100.2, respectively

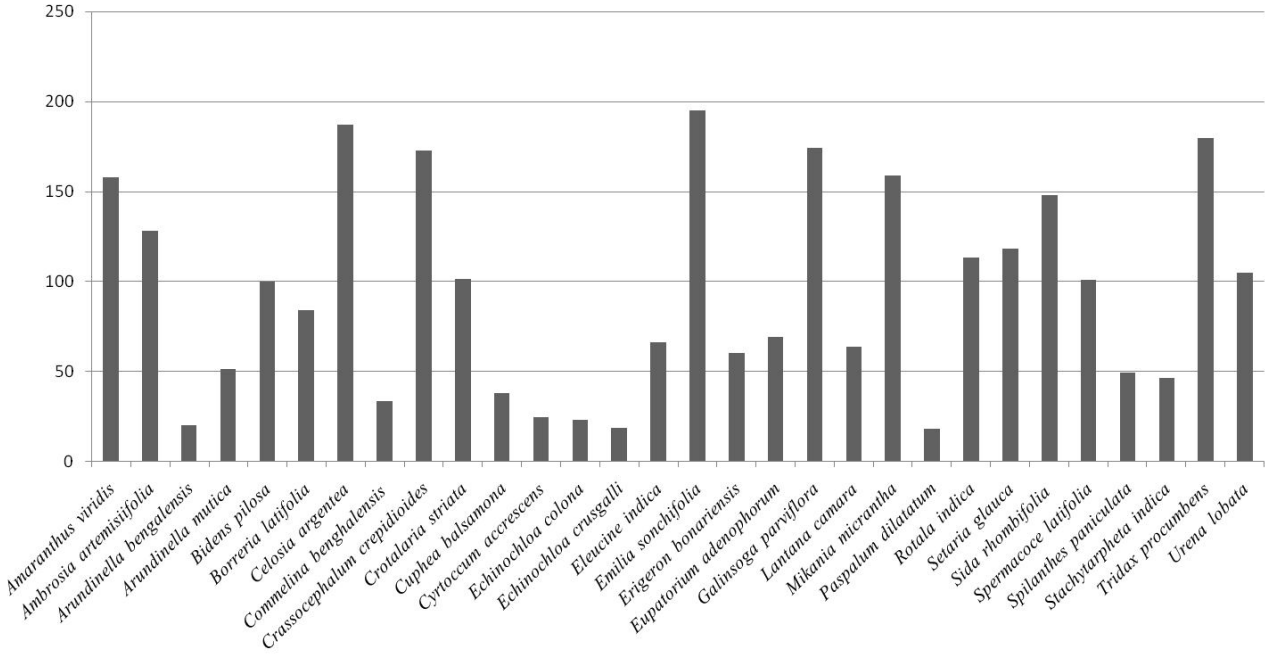

Figure 2. AUDPC used for susceptibility analysis on common weeds with *Rhizoctonia solani* AG 1-IB isolate

and all were statistically at par followed by *B. latifolia* (84.0), *E. adenophorum* (69.4), *E. indica* (66.2), *L. camara* (64.0) and *E. bonariensis* (60.7). The weeds *A. mutica* (51.4), *S. paniculata* (49.7) and *S. indica* (46.7) were statistically at par followed by *C. balsamona* (38.0) and *C. benghalensis* (33.7) which were also statistically at par followed by *C. accrescens* (24.8). The weeds *P. dilatatum* (18.1) *E. crusgalli* (18.6) and *A. bengalensis* (20.1) were least susceptible and statistically at par followed by *E. colona* (23.4). Saveinai *et al*. (2017) have conducted the susceptibility analysis of *R. solani* AG 1-IA on weeds by using AUDPC criteria. They also observed great variation in susceptibility of weeds to *R. solani* AG 1-IA using this criteria.

Most of common weeds in Meghalaya are susceptible to *R. solani* AG 1-IB, but few are highly susceptible. Highly susceptible weeds should not be used as a mulch because this practise will help in spreading this pathogen.

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