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Pathogenic and Genetic Variation among the Isolates of *Rhizoctonia solani* (AG 1-IA), the Rice Sheath Blight Pathogen

Arra Yugander[1], Duraisamy Ladhalakshmi[1], Vellaichamy Prakasham[1], Satendra K. Mangrauthia[2], Madamsetty S. Prasad[1], Donempudi Krishnaveni[1], Maganti Sheshu Madhav[2], Raman M. Sundaram[2] and Gouri Sankar Laha[1]

1 Department of Plant Pathology, Directorate of Rice Research, Hyderabad, India
2 Department of Plant Biotechnology, Directorate of Rice Research, Hyderabad, India

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Correspondence
G.S. Laha, Directorate of Rice Research, Hyderabad, India.
E-mail: lahags@yahoo.co.in

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Abstract

Sheath blight disease of rice caused by *Rhizoctonia solani* is one of the most dreaded plant diseases faced by the rice farmers all over the world. None of the commercially cultivated rice varieties have sufficient level of field resistance, and the disease is presently being managed by chemical pesticides. In this study, 40 isolates of rice sheath blight pathogen, collected from diverse rice ecosystems from 12 different states of India, were characterized for their morphological, pathological and genetic variation. The isolates showed wide morphological variation in terms of size of sclerotia and abundance of sclerotia production. The virulence of each pathogen isolate was studied on four rice varieties, that is TN1, IR 64, Tetep and Swarnadhan in glasshouse, and observations were taken by measuring the relative lesion height. The relative lesion heights produced by these isolates on four different rice varieties varied widely. Genetic variation of the isolates was analysed using ISSR markers. The primers based on AG, GA, AC and CA repeats were informative and revealed polymorphism among the isolates. The polymorphism information content (PIC) of the primers ranged from 0.80 to 0.96, while the resolving power (*Rp*) ranged from 3.7 to 15.35. Largely, grouping of the isolates happened based on their geographical origin. One isolate from Titabar, Assam, and another from Adialabad, Telangana, were quite distinct from rest of the isolates.

Introduction

Sheath blight disease of rice caused by the fungus *Rhizoctonia solani* Kuhn [teleomorph: *Thanetophorus cucumeris* (Frank) Donk] is one of the most dreaded rice diseases which limit its production. Due to changed cultivation practices which include widespread cultivation of few high-yielding rice varieties (narrow genetic base), heavy dependency on chemical fertilizers and apparent changes in the climate, there has been increased incidence of this disease in India (DRR 1975–2013). The data from Production Oriented Survey (DRR 1975–2013) on rice shows that the intensity of the disease has increased over the years and many areas where it was absent or sporadic are showing widespread occurrence of the disease.

Various estimates of crop losses due to sheath blight ranging from negligible to 50% have been reported (Lee and Rush 1983). The fungus has a wide host range and can infect plants belonging to 32 families and 188 genera (Gangopadhyay and Chakrabarti 1982). On the basis of different anastomosis reactions, the isolates of *Rhizoctonia* are classified into 14 anastomosis groups (AGs) (Carling et al. 2002; Mikhail et al. 2010). Within anastomosis groups, intraspecific groups (ISGs) are recognized. The rice sheath blight fungus belongs to anastomosis group 1 IA (AG 1-IA).

Although AG grouping gives a broad idea regarding the host specificity of *Rhizoctonia* isolates, within AG group, there exists a tremendous amount of variation in terms of their genetic make-up, morphology (abundance of sclerotia production, sclerotial size), pathogenicity and aggressiveness. Earlier studies have revealed high genetic variation among the isolates of rice sheath blight pathogen (Banniza and Rutherford 2001; Taheri et al. 2007). Although information on differential reaction of *R. solani* isolates on a standard set of cultivars is limited, variation in virulence among the isolates of *R. solani* has been reported (Tsai 1973; Haque 1975; Reddy 1991; Huang et al. 2011). Variation in the isolates of the pathogen has been measured based on morphological characteristics (Vijayan and Nair 1985), isoenzyme variation (Neeraja et al. 2002a,b; Mohammadi et al. 2003), fatty acid composition (Stevens Johnk and Jones 1994) and using various molecular markers like restriction fragment length polymorphism (RFLP) (Banniza et al. 1999; Rosewich et al. 1999), amplified fragment length polymorphism (AFLP) (Taheri et al. 2007; Fan et al. 2008), rep-PCR (Linde et al. 2005), random amplified polymorphic DNA (RAPD) (Neeraja et al. 2002a,b) and rDNA-internal transcribed spacer (Sharon et al. 2006). Hu et al. (2005) found significant correlation between protein patterns and RAPD banding patterns among the isolates of sheath blight pathogen which indicated that the variation in pathogenicity was linked to the hereditary differentiation of this fungal pathogen. Inter Simple sequence repeat–PCR (ISSR–PCR) is a simple, cost-efficient, robust, multilocus marker system which has been used in determining genetic variability among fungal pathogens of crop plants (Menzies et al. 2003; Chadha and Gopalakrishna 2007). In this study, ISSR-PCR was used in studying the genetic variability among *R. solani* isolates collected from different rice-growing regions of India.

Materials and Methods

Isolation of the pathogen, pathogenicity, morphological characters and maintenance of the pathogen

Leaf sheath or leaves showing typical sheath blight symptoms were collected from different rice-growing regions of India (Table 1). The infected leaf sheath or leaf pieces were initially washed in running tap water, cut into 5-mm pieces, treated with 0.1% mercuric chloride for 30 s, washed three times with sterile distilled water, blotted dry and then placed on 2% plain agar. The plates were then incubated for 24–48 h at 27°C. Hyphal tips of mycelium with morphological characteristics typical of *R. solani* growing out from the leaf or sheath pieces were subcultured onto potato dextrose agar (PDA). A list of the isolates used in this study is given in Table 1. The isolates were maintained in PDA slants at 4°C. For observations on morphological characters, each isolate was grown by inoculating a single sclerotium of the fungus in the centre of PDA plates (90-mm-diameter petriplate). Abundance of sclerotia production was recorded visually, and sclerotial diameter was measured with the help of a thickness gauge (Mitutoyo, Japan). The pathogenicity of the isolates was confirmed by detached cut-leaf technique (Dath 1987).

Virulence analysis

To study the virulence spectrum of *R. solani* isolates, each isolate was inoculated on a set of four rice varieties having varying degree of tolerance to sheath blight disease, *viz*. Taichung Native 1 (TN1) (susceptible), IR 64 (moderately susceptible), Tetep and Swarnadhan (both moderately resistant). The rice varieties were grown in earthen pots (12 inches diameter), and the plants at the maximum tillering stage were used for inoculation. In each earthen pot, three plants were raised and each pot was taken as one replication. For each isolate variety combination, three replications were maintained. The isolates of *R. solani* were grown for 10 days on autoclaved Typha stem cutting (*Typha angustata*; an aquatic weed) soaked with a solution containing 2% sucrose and 1% peptone (Bhaktavatsalam et al. 1978). Plant inoculation with individual *R. solani* isolates was carried out at the maximum tillering stage by placing 4–5 Typha stem cuttings colonized with the pathogen inside each hill (Bhaktavatsalam et al. 1978). The inoculated plants were kept in a humidity chamber for 4–5 days and then shifted to normal glasshouse benches. Observations were taken 2 weeks after inoculation by measuring the relative lesion height (RLH) following Vidhyasekaran et al. (1997) where RLH in each tiller was calculated as RLH (%) = [highest point a lesion is seen (cm) × 100]/ [plant height (cm)] and then making the average.

DNA extraction, PCR details and gel electrophoresis

DNA was isolated following the method of George et al. (1998). The isolates were grown in potato dextrose broth (PDB) under still culture at 27°C. After 72 h of growth, when sclerotial initials were visible,

Table 1 Details of *Rhizoctonia solani* isolates used in the study

R. solani isolates	Source place/state	Rice variety from which isolated	Abundance/distribution of sclerotia in culture plate	Mean sclerotia diameter (mm) ± SE
Lud-1	Ludhiana/Punjab	PAU-201	Moderate, well distributed	1.57 ± 0.23
Lud-2	Ludhiana/Punjab	PAU-201	Moderate, around periphery	1.29 ± 0.19
Kpt-1	Kapurthala/Punjab	PR 116	Less, well distributed	1.49 ± 0.14
Kul-1	Kaul/Haryana	TN1	Moderate, around periphery	1.00 ± 0.11
Kul-2	Kaul/Haryana	Taraori Basmati	Moderate-high, around periphery	1.01 ± 0.04
Mrt-1	Meerut/Uttar Pradesh	Unknown	Abundant, well distributed	1.30 ± 0.18
Ngn-1	Nagina/Uttar Pradesh	Unknown	Less, well distributed	1.34 ± 0.21
Pnt-1	Pantnagar/Uttarakhand	Pant Dhan-4	Abundant, well distributed	1.74 ± 0.24
Rpr-1	Raipur/Chhatishgarh	Swarna	Abundant, well distributed	1.18 ± 0.26
Rpr-2	Raipur/Chhatishgarh	Swarna	Less-moderate, distributed	1.41 ± 0.23
Ttb-1	Titabar/Assam	Rasi	Moderate-high, around periphery	1.17 ± 0.21
Ttb-2	Titabar/Assam	Basundhara	Moderate, around periphery	1.24 ± 0.15
Ttb-3	Titabar/Assam	Satyaranjan	Abundant, well distributed	1.86 ± 0.56
Ttb-4	Titabar/Assam	Gitesh	Abundant, well distributed	1.62 ± 0.32
Gra-1	Gerua/Assam	Chandrama	Abundant, well distributed	0.84 ± 0.16
Gra-2	Gerua/Assam	Breeding lines	Abundant, well distributed	1.66 ± 0.38
Gra-3	Gerua/Assam	Weeds	Abundant, well distributed	1.30 ± 0.34
Gra-4	Gerua/Assam	Chandrama	Abundant, well distributed	1.57 ± 0.44
Imph-1	Imphal/Manipur	TN1	Less, around periphery	1.92 ± 0.30
Imph-2	Imphal/Manipur	TN1	Moderate, around periphery	0.97 ± 0.08
Lpt-1	Lamphalpet/Manipur	Breeding lines	Moderate, around periphery	2.16 ± 0.61
Lpt-2	Lamphalpet/Manipur	Unknown	Abundant, around periphery	1.59 ± 0.46
Isb-1	Iroisemba/Manipur	Leimaphou	Less, well distributed	1.55 ± 0.32
Lchr-1	Lembuchera/Tripura	Unknown	Moderate, around periphery	1.64 ± 0.20
Adn-1	Arundhatinagar/Tripura	Swarna	Abundant, well distributed	1.02 ± 0.12
Adn-2	Arundhatinagar/Tripura	Swarna	Moderate, around periphery	1.94 ± 0.58
Adn-3	Arundhatinagar/Tripura	TN1	Abundant, well distributed	1.64 ± 0.44
Bpn-2	Barapani/Meghalaya	Unknown	Moderate, around periphery	1.51 ± 0.26
Bpn-3	Barapani/Meghalaya	Breeding lines	Abundant, well distributed	1.57 ± 0.39
Bpn-4	Barapani/Meghalaya	Breeding lines	Abundant, well distributed	1.58 ± 0.38
Chn-1	Chinsurah/West Bengal	Swarna	Abundant, around periphery	1.07 ± 0.18
Chn-2	Chinsurah/West Bengal	TN1	Moderate, around periphery	1.32 ± 0.20
Rnr-1	Rajnedranagar/Telangana	TN1	Abundant, well distributed	1.30 ± 0.16
Rnr-2	Rajendranagar/Telangana	HR-12	Abundant, around periphery	1.14 ± 0.18
Rnr-3	Rajendranagar/Telangana	TN1	Moderate-high, well distributed	1.65 ± 0.37
Adb-1	Adilabad/Telangana	Samba Mahsuri	Moderate, well distributed	2.19 ± 0.57
Adb-2	Adilabad/Telangana	Samba Mahsuri	Less, well distributed	2.08 ± 0.58
Wgl-1	Warangal/Telangana	WGL-14	Abundant, well distributed	1.60 ± 0.33
Wgl-2	Warangal/Telangana	Vijaya Mahsuri	Abundant, well distributed	1.87 ± 0.41
Mnd-1	Mandya/Karnataka	Jyothi	Less, mainly in the centre	1.17 ± 0.35

Low: <100 sclerotia/plate; Moderate: 100–200 sclerotia/plate; Abundant: >200 sclerotia/plate.

the mycelial mat was harvested, washed with sterile distilled water repeatedly and then squeezed in between layers of sterile blotting papers to remove any excess water. The mycelial mat was frozen in liquid nitrogen and ground into fine powder. In some cases, mycelial mat was directly ground into paste in a pestle and mortar (coarse type). Approximately 30–40 mg of mycelial powder or paste was suspended in 650 µl of extraction buffer (100 mM Tris, pH 8; 100 mM EDTA; 250 mM NaCl; and 1% sodium dodecyl sulphate, wt/vol), incubated at 65°C for at least 30–45 min. Cellular proteins were precipitated with 100 µl of potassium acetate (3 M potassium and 5 M acetate, pH 4.8), and then, DNA was precipitated using isopropanol. The DNA was dissolved in 100 µl of sterile distilled water, and its quality was checked through electrophoresis. Two microlitres of DNA solution (50 ng) was used as template for PCR. The sequence details of the ISSR primers were obtained from University of British Columbia website. Initially, 50 primers (UBC 801-UBC 850) were screened with a subset of samples. Eighteen primers which gave scor-

able banding pattern were used for analysis of all the samples. A single primer was used at a time for all the samples. Each reaction mix of 20 µl contained 2 µl of genomic DNA (50 ng), 1 µl of primer (5 mM primer solution), 2 µl of 10× buffer (0.1 M *Tris* pH 8.3; 0.5 M KCl; 7.5 mM $MgCl_2$; 0.1% gelatin), 1 µl of 2.5 mM dNTPs and 1.0 unit of Taq polymerase. An additional 1 µl of $MgCl_2$ (25 mM concentration) was added for better performance. PCR amplifications were carried out in a thermal cycler (Applied Bio-systems, USA) with the following conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 7 min. The amplification products were mixed with loading buffer (40% sucrose and 0.25% bromophenol blue) and resolved in 2% agarose gel in 1X TBE buffer under room temperature at a constant voltage of 90 V and detected by ethidium bromide staining. The molecular weight marker, 1Kb ladder (Bangalore Genei Private Limited, India), was used for band sizing.

Preparation of dendrogram and calculation of primer parameters

Each amplification product/band was considered as an ISSR marker allele. The reproducibility of the DNA profiles for all the isolates and for all the selected primers was tested by repeating the PCR, and only reproducible amplicons were considered for analysis. Amplicons were recorded as present (1) or absent (0). The data were analysed for calculation of various parameters like number of loci, number of polymorphic loci, polymorphism (%), polymorphism information content (PIC) and primer resolving power (*Rp*). The PIC values were calculated based on the following formula

$$\mathrm{PIC} = 1 - \sum_{j=1}^{n} Pij2$$

where *Pij* = frequency of the jth pattern of the ith band. Alternatively, PIC was calculated using online PIC calculator software (http://www.genomics.liv.ac.uk/animal/Pic1.html). Primer resolving power (*Rp*) was calculated according to Prevost and Wilkinson (1999) as follows

$$Rp = \sum Ibi, \text{ where } Ibi = 1 - (2 \times |0.5 - p_i|)$$

where *Ibi* is the informativeness of the *i*th band and p_i is the proportion of accessions containing the ith band.

All the numerical and taxonomical analyses were conducted using NTSYS–PC software (version 2.02 from Exeter software, NY; Rohlf 1998). Dice similarity coefficient values for 780 pairwise comparisons between isolates were calculated, and a similarity coefficient matrix was constructed using SIMQUAL subroutine. Matrices of similarity coefficients were then subjected to unweighted pair group method using arithmetic average analysis (UPGMA) to generate a dendrogram using the SAHN subroutine and Tree plot of NTSYS-PC.

Results

Forty isolates of rice sheath blight fungus *R. solani* were obtained from infected samples collected from 12 different states of India. The details of the isolates collected and used in this study are presented in Table 1. These isolates produced typical sheath blight symptoms on detached cut leaves of rice variety TN1. The isolates showed wide morphological variation in terms of size of sclerotia and abundance of sclerotia production. The isolates like Rnr-2, Adn-1 and Gra-1 produced very large number of sclerotia (>400 sclerotia/plate), while some isolates like Imph-1, Mnd-1 and Ngn-1 produced very less sclerotia (20–40 sclerotia/plate). Sclerotial diameter varied from 0.84 mm (Gra-1) to 2.19 mm (Adb-1). Some isolates like Ttb-3 and Wgl-2 produced abundant number of large-sized sclerotia (1.86–1.87 mm diameter). The distribution of the sclerotia in the culture plate also differed among the isolates. While some of the isolates produced sclerotia in a circular fashion around the periphery of the plate, in others, the sclerotia were distributed all over the plate (Table 1).

The relative lesion height (RLH) data of all the isolates are presented in Table 2. All the 40 isolates of the pathogen produced typical sheath blight symptoms on all four rice varieties. However, the intensity of the disease varied depending on the isolates and varieties. The RLH on TN1 ranged from 29% to 72.67% and on IR 64 from 25% to 72.67%. The RLHs were comparatively less on other two varieties. Considering the reaction on all the four varieties, the isolates were categorized as highly virulent (average RLH >45%), moderately virulent (average RLH 35–45%) and less virulent (average RLH <35%). The isolates like Lud-1, Rpr-2, Chn-1, Pnt-1, Rnr-2, Rnr-3, Gra-2, Gra-3, Gra-4, Lpt-2, Ttb-4, Adn-2, Adn-3, Adb-2 and Wgl-2 were categorized as highly virulent, while Imph-1, Imph-2 and Chn-2 were less virulent. Rest of the isolates were moderate in virulence (Table 2).

Table 2 Relative lesion heights on different rice varieties having different levels of tolerance to sheath blight disease

Isolates	Relative lesion height (%)				Virulence category
	TN1	IR64	Tetep	Swarnadhan	
Lud-1	56.01 (68.33) ab	40.18 (41.67) ghi	39.79 (41.00) def	39.79 (41.00) abcdef	HV
Lud-2	50.56 (59.33) bcdefghi	39.59 (40.67) hi	40.37 (42.00) cdef	28.86 (23.33) mn	MV
Kpt-1	49.98 (58.67) bcdefghij	37.23 (36.67) i	40.38 (42.00) cdef	32.48 (29.00) ghijklm	MV
Kul-1	40.18 (41.67) lmnop	38.82 (39.33) i	40.38 (42.00) cdef	37.45 (37.00) defgh	MV
Kul-2	39.02 (39.67) nop	39.41 (40.33) hi	38.82 (39.33) defg	28.63 (23.00) mn	MV
Mrt-1	50.76 (60.00) bcdefgh	39.59 (40.67) hi	39.41 (40.33) defg	36.26 (35.00) efghij	MV
Ngn-1	52.32 (62.67) bcdef	30.19 (25.33) j	30.40 (25.67) k	43.25 (47.00) abc	MV
Pnt-1	50.37 (59.33) bcdefghij	51.93 (62.00) bc	39.99 (41.33) cdef	37.44 (37.00) defgh	HV
Rpr-1	58.53 (72.67) a	40.38 (42.00) ghi	37.04 (36.33) efghij	30.63 (26.00) jklm	MV
Rpr-2	49.78 (58.33) cdefghijk	58.67 (72.67) a	38.42 (38.67) defgh	38.24 (38.33) cdef	HV
Ttb-1	51.35 (61.00) bcdefgh	37.84 (37.67) i	30.63 (26.00) jk	29.09 (23.67) mn	MV
Ttb-2	45.56 (51.00) hijkl	31.42 (27.33) j	39.99 (41.33) cdef	38.63 (39.00) cdef	MV
Ttb-3	45.36 (50.67) hijklm	46.52 (52.67) def	39.60 (40.67) defg	29.55 (24.67) lmn	MV
Ttb-4	46.55 (52.67) fghijk	56.36 (69.33) ab	52.32 (62.67) a	45.36 (50.67) a	HV
Gra-1	50.00 (58.67) bcdefghij	38.41 (38.67) i	31.71 (27.67) ijk	28.18 (22.33) mn	MV
Gra-2	53.25 (64.00) abcd	48.84 (56.67) cdef	46.36 (52.33) abc	29.68 (25.00) lmn	HV
Gra-3	49.59 (58.00) defghijk	47.28 (54.00) cdef	40.18 (41.67) cdef	38.40 (38.67) cdef	HV
Gra-4	44.41 (49.00) jklmn	44.03 (48.33) fgh	51.35 (61.00) a	42.68 (46.00) abcd	HV
Imph-1	38.23 (38.33) opq	30.42 (25.67) j	31.27 (27.00) jk	29.93 (25.00) klmn	LV
Imph-2	37.23 (36.67) pq	29.98 (25.00) j	37.84 (37.67) defghi	37.64 (37.33) cdefg	LV
Lpt-1	46.52 (52.67) fghijk	49.98 (58.67) cde	40.18 (41.67) cdef	31.93 (28.33) hijklm	MV
Lpt-2	44.41 (49.00) jklmn	45.37 (50.67) efg	39.20 (40.00) defg	39.55 (40.67) bcdef	HV
Isb-1	50.39 (59.33) bcdefghij	39.99 (41.33) hi	37.84 (37.67) defghi	34.84 (32.67) fghijkl	MV
Lchr-1	37.84 (37.67) opq	37.23 (36.67) i	38.60 (39.00) defg	35.65 (34.00) efghij	MV
Adn-1	49.59 (58.00) defghijk	38.43 (38.67) i	40.18 (41.67) cdef	35.44 (33.67) fghijk	MV
Adn-2	46.13 (52.00) ghijkl	50.19 (59.00) cde	38.62 (39.00) defg	41.11 (43.33) abcde	HV
Adn-3	51.25 (60.67) bcdefgh	48.06 (55.33) cdef	52.13 (62.33) a	44.98 (50.00) ab	HV
Bpn-2	39.01 (39.67) nop	38.20 (38.33) i	40.37 (42.00) cdef	36.25 (35.00) efghij	MV
Bpn-3	46.90 (53.33) efghijk	46.52 (52.67) def	44.03 (48.33) bcd	28.04 (22.67) mn	MV
Bpn-4	32.45 (29.00) q	37.44 (37.33) i	35.68 (34.33) fghijk	24.30 (17.00) n	MV
Chn-1	47.28 (54.00) defghijk	48.64 (56.33) cdef	37.82 (37.67) defghi	36.86 (36.00) efghi	HV
Chn-2	39.39 (40.33) mnop	38.62 (39.00) i	32.14 (28.33) hijk	28.40 (22.67) mn	LV
Rnr-1	51.73 (61.67) bcdefg	39.60 (40.67) hi	30.20 (25.33) k	28.86 (23.33) mn	MV
Rnr-2	50.75 (60.00) bcdefgh	46.34 (52.33) def	40.57 (42.33) cdef	37.25 (36.67) defghi	HV
Rnr-3	48.84 (56.67) defghijk	50.36 (59.33) cde	43.06 (46.67) bcde	31.72 (28.00) ijklm	HV
Adb-1	44.41 (49.00) jklmn	46.32 (52.33) ef	33.34 (33.33) ghijk	39.79 (41.00) abcdef	MV
Adb-2	44.60 (49.33) ijklmn	49.59 (58.00) cde	40.57 (42.33) cdef	37.45 (37.00) defgh	HV
Wgl-1	55.76 (68.33) abc	52.34 (62.67) bc	43.06 (46.67) bcde	29.30 (24.33) lmn	MV
Wgl-2	52.94 (63.67) abcde	51.54 (61.33) bcd	49.43 (57.67) ab	42.68 (46.00) abcd	HV
Mnd-1	43.80 (48.00) klmno	39.01 (39.67) hi	40.76 (42.67) cdef	37.64 (37.33) cdefg	MV
CV (%)	7.95	7.44	10	9.88	
LSD (5%)	6.0855	5.2022	6.4347	5.6384	

Data were arc sine transformed before analysis. Figures in the parentheses indicate original means; figures in a column with same letter indicate that they do not differ significantly; HV, high virulence; MV, moderate virulence; LV low virulence.

Of 50 ISSR primers tested, scorable and reproducible banding patterns were obtained from 18 primers. The details of primerwise parameters are presented in Table 3. Fig. 1 shows a representative banding pattern of selected isolates of *R. solani* using the primer UBC 807. Eighteen primers produced 329 band positions (loci), and all were polymorphic. Majority of the primers which produced polymorphic bands among the isolates of *R. solani* were based on AG or GA repeats followed by AC or CA repeats. Among the primers used, UBC-841 [$(GA)_8YC$] produced maximum number of loci (28), while UBC-816 [$(CA)_8T$] produced least number of loci (9) (Table 3). The polymorphism (%) of all the primers was 100%. The PIC

Table 3 Details of the primers, polymorphism and banding patterns of 24 isolates of *Rhizoctonia solani* by 18 ISSR primers

ISSR primers (UBC)	Primer sequence	No. of loci	No. of polymorphic loci	Polymorphism (%)	PIC	*Rp*	Mean genetic similarity
807	$(AG)_8T$	23	23	100.00	0.94	11.45	0.65
808	$(AG)_8C$	25	25	100.00	0.95	10.35	0.74
809	$(AG)_8G$	23	23	100.00	0.94	10.25	0.70
810	$(GA)_8T$	16	16	100.00	0.87	6.15	0.52
811	$(GA)_8C$	10	10	100.00	0.87	6.35	0.50
812	$(GA)_8A$	15	15	100.00	0.90	7.9	0.49
816	$(CA)_8T$	9	9	100.00	0.80	3.7	0.55
817	$(CA)_8A$	10	10	100.00	0.86	5.45	0.57
825	$(AC)_8T$	20	20	100.00	0.94	12	0.56
826	$(AC)_8C$	17	17	100.00	0.92	8.25	0.66
827	$(AC)_8G$	26	26	100.00	0.94	12.65	0.60
834	$(AG)_8YG$	16	16	100.00	0.93	6.5	0.76
835	$(AG)_8YC$	17	17	100.00	0.93	10.9	0.62
836	$(AG)_8YA$	22	22	100.00	0.94	10.95	0.61
840	$(GA)_8YT$	16	16	100.00	0.92	7.55	0.68
841	$(GA)_8YC$	28	28	100.00	0.96	15.35	0.63
842	$(GA)_8YG$	17	17	100.00	0.91	5.7	0.74
847	$(CA)_8RC$	19	19	100.00	0.93	11.3	0.42

Y = (C,T); R = (A,G).

value ranged from 0.80 (UBC 816) to 0.96 (UBC-841). Most of the 3-prime single-base anchored AG and AC repeats had higher PIC values (0.92–0.95) compared to 3-prime single-base anchored CA and GA repeats (0.80–0.90). The primers 807, 825, 827, 835, 836 and 847 were more informative with higher PIC and *Rp* values and with corresponding lower mean genetic similarity values (Table 3). All these six primers individually could distinguish 34–38 of 40 isolates of *R. solani* with distinct profile. Interestingly, primers 808 and 809 which are having higher PIC and *Rp* values but comparatively higher mean genetic similarity also could distinguish 38 and 36 isolates, respectively, of 40.

A dendrogram (Fig. 2) was generated using pooled ISSR data (18 primers, 329 loci, 7031 bands) which divided 40 *R. solani* isolates into 6 major clusters at 66% similarity. The isolates Gra-1 from the state of Assam (mean genetic similarity 0.55), Adb-2 from the state of Telangana (mean genetic

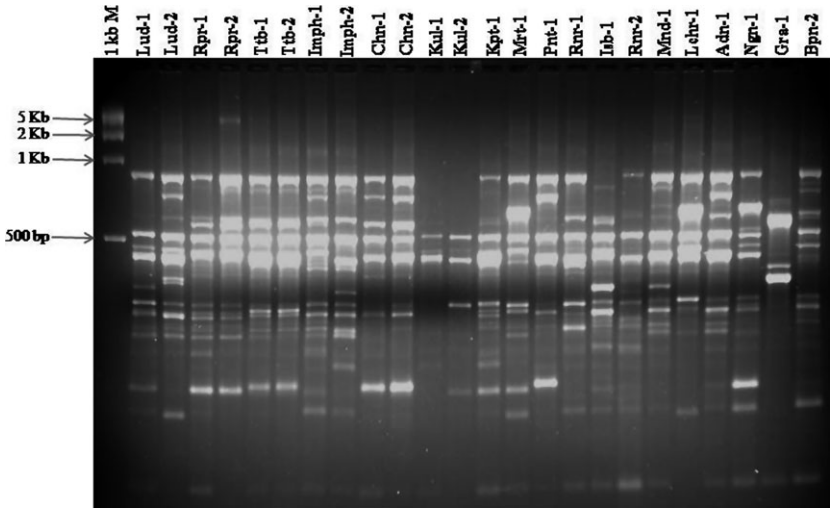

Fig. 1 Electrophoretic banding pattern of selected isolates of *Rhizoctonia solani* using primer UBC-807 [$(AG)_8T$].

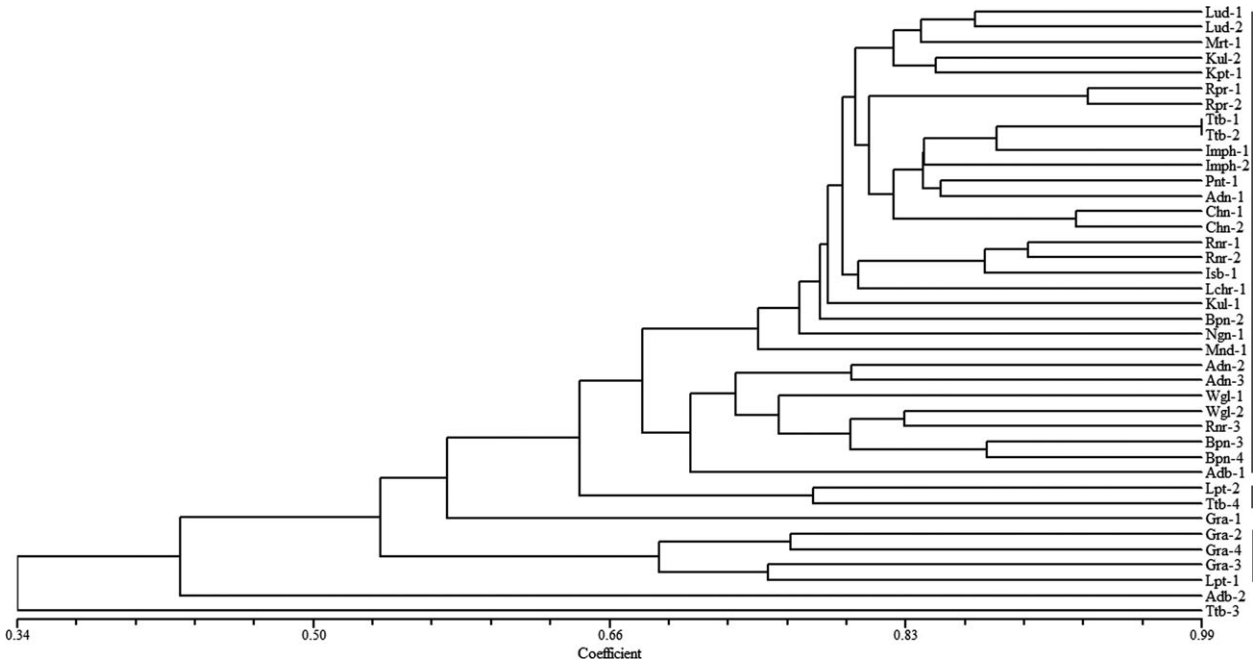

Fig. 2 UPGMA dendrogram showing clustering of 40 isolates of *Rhizoctonia solani*. It is derived from Dice similarity coefficient matrix of 780 pairwise comparison using pooled ISSR (18 primers, 329 loci, 7031 bands) data.

similarity 0.42) and Ttb-3 from the state of Assam (mean genetic similarity 0.34) were quite distinct from other isolates and also from each other. These isolates were also morphologically different from other isolates (Table 1). In the major cluster, the isolates Kul-1 (genetic similarity 0.70), Bpn-2 (genetic similarity 0.69), Ngn-1 (genetic similarity 0.68), Mnd-1 (genetic similarity 0.66) and Adb-1 (genetic similarity 0.62) were also quite different from rest of the members of the cluster. In general, the isolates from same or neighbouring places grouped together (Fig. 2). Higher intraregional genetic variation was observed in case of isolates from Assam (47%), Telangana (37%), Manipur (33%), Tripura (27%) and Meghalaya (24%).

In the main cluster, the isolates from north Indian states of Punjab, Haryana and Uttar Pradesh (Lud-1, Lud-2, Mrt-1, Kul-2 and Kpt-1), central Indian state of Chhattishgarh (Rpr-1 and Rpr-2), eastern and north-eastern states like Assam, Manipur, Tripura and West Bengal (Ttb-1, Ttb-2, Imph-1, Imph-2, Adn-1, Chn-1 and Chn-2), and south Indian state of Telangana (Rnr-1 and Rnr-2) formed distinct separate subclusters. The isolates from south Indian states of Telangana and Karnataka (Mnd-1, Wgl-1, Wgl-2, Rnr-3 and Adb-1) formed another subcluster. However, the isolates like Pnt-1 (Uttarakhand, north India) grouped with isolates from eastern and north eastern states and the isolates like Isb-1 (Manipur, north eastern India), Lchr-1 (Tripura, north eastern India) and Bpn-2 (Meghalaya, North eastern India) grouped with North western Indian isolates like Kul-1 (Haryana) and Ngn-1 (western Uttar Pradesh). The remaining isolates from north eastern India like Gra-1-4 and Ttb-4 (Assam), Lpt-1 and 2 (Manipur) grouped together.

Discussion

Sheath blight is an important disease of rice in India. The fungus shows tremendous variation in terms of their morphological characteristics. A number of reports have shown variation among the geographically different isolates of the pathogen in terms of abundance of sclerotia, sclerotial size and colour and colour of the mycelium (Sherwood 1969; Parmeter and Whitney 1970; Manian 1982; Zou et al. 2011). Large variation in size, number and type of sclerotia on agar media has been reported by several studies (Tsai 1973; Manian 1982). In the present study also, we recorded a wide variation among different isolates in terms of size and number of sclerotia. In general, in most of the isolates which produced less number of sclerotia, the sclerotial diameter was more. However, some isolates of the pathogen like Ttb-3 and Wgl-2 produced abundant number of large-sized sclerotia.

The virulence profile of *R. solani* isolates was studied on four rice varieties having difference in their susceptibility level. There is no resistant variety against sheath blight disease of rice. The rice varieties Swarnadhan (IET 5656) and Tetep show moderate resistance (Sha and Zhu 1990; Rani et al. 2008). The rice variety IR 64 shows moderate susceptibility (Zuo et al. 2009). The RLH on these varieties varied considerably among different isolates. Although comparatively lower RLH was recorded in Tetep and Swarnadhan, differences in RLH between TN1 and IR 64 were not prominent. Based on the average RLH on these rice varieties, the isolates were categorized as highly virulent, moderately virulent and less virulent. Isolates differing in virulence have also been reported by different workers (Tsai 1973; Haque 1975; Reddy 1991; Xiao et al. 2008).

Studying the variation in the pathogen is important from the point of epidemiology and management of the disease. Moreover, variability information also helps researchers to breed a suitable disease-resistant rice variety. A number of studies suggest that sheath blight pathogen is highly diverse than it was previously thought (Vilgalys and Hester 1990; Liu and Sinclair 1993). As typical host differential system for characterizing the *R. solani* isolates is not yet fully standardized, molecular tools have been used for characterizing the genetic variability in this fungus. A number of biochemical and molecular markers have been used for studying the variation in the fungus. ISSR–PCR is a simple, cost-efficient, robust, multilocus marker method for determining genetic variability among the germplasm accessions/isolates of pathogens. Although there are lots of reports of use of ISSRs for studying diversity of crop germplasm, its use in pathogen diversity analysis is not very common. In this study, we have used ISSR-PCR to characterize different isolates of *R. solani*. Of 50 ISSR primers screened, 18 primers produced scorable and reproducible banding pattern. The number of loci varied from 9 (primer 816) to 28 (primer 841). Most of these primers which produced polymorphic bands were based on AG or GA repeats followed by AC or CA repeats. In an earlier study on analysis of genetic variation among the isolates of *Magnaporthe grisea* using ISSRs, Chadha and Gopalakrishna (2007) have also found that AG or GA repeats were more useful in differentiating the fungal isolates. Both PIC and *Rp* are important in determining the value of a primer to distinguish genotypes. The primers 807, 825, 827, 835, 836 and 847 exhibited higher PIC and *Rp* values and corresponding lower mean genetic similarity values and were more informative (Table 3). All these six primers individually could distinguish 34–38 of 40 isolates of *R. solani* with distinct profile.

Dendrogram generated from the pooled ISSR data (18 primers, 329 loci, 7031 bands) clearly grouped the isolates based on their geographical origin rather than virulence groups. This also indicates that isolates belonging to same group could show great genetic variability. However, some isolates categorized as high virulence viz., Lpt-2, Ttb-4, Gra-2, Gra-3, Gra-4 and Adb-2 grouped closely. Similarly, although there was no strong correlation between any morphological characters and ISSR groupings, the isolates like Adn-2, Adn-3, Wgl-1, Wgl-2, Rnr-3, Bpn-3, Bpn-4, Lpt-2, Ttb-4, Gra-1-4 and Lpt-1 which produced abundant number of sclerotia grouped closely. The use of more number of loci from different ISSR primers increases the accuracy of grouping of the isolates. The isolates Gra-1 from the state of Assam (mean genetic similarity 0.55), Adb-2 from the state of Telangana (mean genetic similarity 0.42) and Ttb-3 from the state of Assam (mean genetic similarity 0.34) were quite distinct from other isolates and also from each other. The isolate Gra-1 produced large number (>400) of smaller-size sclerotia in each plate. In the main cluster, the isolates from North India, central India, east and north east India and south India formed distinct subcluster. However, the isolate Pnt-1, from Pantnagar (Uttarakhand, North India), showed more closeness to the isolates from east and north east India. Similarly, some isolates from north eastern parts of India like Adn-2 and Adn-3 (Tripura, North east India) and Bpn-3 and Bpn-4 (Meghalaya, North east India) were more similar to isolates from south India. Similar results were reported by Zhou et al. (2002) who found that DNA polymorphism was clearly correlated with geographical origins with no obvious correlation with pathogenicity. Yi et al. (2002) also did not find any correlation between virulence and RAPD groupings.

The study reveals that a large variation exists among rice-infecting strains of *R. solani* in India. Fingerprinting of the isolates using selected ISSRs along with morphological characterization and virulence analysis will help epidemiological studies that can provide new insights into pathogen biology and disease spread.

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