

## Original Research Article

<https://doi.org/10.20546/ijcmas.2018.710.080>

## Variability Studies on Sheath Blight of Rice in Karnataka, India

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### ABSTRACT

#### Keywords

*Rhizoctonia solani* Kühn  
[Teleomorph:  
*Thanatephorus cucumeris*  
(Frank) Donk]

#### Article Info

**Accepted:**  
06 September 2018  
**Available Online:**  
10 October 2018

Variability in 20 isolates of *Rhizoctonia solani* Kühn [Teleomorph: *Thanatephorus cucumeris* (Frank) Donk] causing sheath blight of rice and weeds, was observed by RAPD and ISSR DNA analysis in conjunction with morphology and pathogenicity studies. Studies on morphological characterization of *R. solani* isolates showed that isolates were highly variable in mycelial growth, color and sclerotial parameters. Pathogenicity of *R. solani* was also evaluated and based on their virulence, isolates were classified highly virulent. Genetic diversity of *R. solani* isolates from different locations using three RAPD and ISSR markers showed less polymorphism at DNA level. The PIC value ranged from 0.82 to 0.88. Relation between cultural/morphological characteristics, Pathogenicity and genetic variation based on RAPD and ISSR markers, and possible reasons for high intra-group variability are discussed.

### Introduction

Sheath blight of rice caused by *Rhizoctonia solani* [Teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is one of the major biotic constraints in India and reduce rice yield ranging from 20-50% depending on the severity of the disease and stages of infection. The disease has spread widely in terms of both occurrence and intensity over past 20 years. At present it is one of the major production constraints in the states of Punjab, Haryana, Uttarakhand, Eastern Uttar Pradesh, Bihar, West Bengal, Odisha, Chhattishgarh, coastal areas of Andhra Pradesh, Tamil Nadu and Kerala and parts of Karnataka. The *R. solani*

emerged as an economically important rice pathogen, due to the intensification of rice-cropping systems with the development of new dwarf, high-tillering, high yielding varieties, high plant densities and an increase in nitrogen fertilization. These factors promote disease spread by providing a favorable microclimate, due to a denser leaf canopy with an increased leaf-to leaf and leaf-to-sheath contact. Breeding for sheath blight resistant cultivars has been a priority area in India. However lack of resistant donors, non-availability of markers and lack of adequate information on the genetic variability of fungal population are some of the limiting factors for developing suitable strategies for

control measures (Neeraja *et al.*, 2002). Further, the fungus has worldwide distribution (Ogoshi, 1987) and isolates of *R. solani* are highly variable in aggressiveness. Although earlier studies suggested that AG-1 IA represented a homogenous group of *R. solani* (Kuninga and Yokosawa, 1982), recent investigations support the hypothesis that the sheath blight pathogen is far more diverse than previously assumed (Neeraja *et al.*, 2002; Singh *et al.*, 2002; Yu *et al.*, 2003; Susheela *et al.*, 2004). Bridge *et al.*, (1995) concluded that integration of genetic techniques with functional characters could provide a powerful tool for characterization of fungal plant pathogens, particularly in respect of host and cultivar-specific populations such as weeds.

## Materials and Methods

### Isolation of the pathogen, pathogenicity and maintenance of the pathogen

Leaves or leaf sheath showing characteristic sheath blight symptom were collected from major rice growing areas of Karnataka, India (Table 1). Infected plant tissues are cut into pieces of 5 cm, washed in running tap water to eliminate any attached organic debris and blotted dry. Small samples of plant tissue (0.5 cm of length) are then cut from the lesions and transferred to an isolation medium i.e., 2% water agar. The plates are then incubated for 24-48 hrs at 28-30 °C. Mycelial tips with morphological characters typical of *R. solani*, growing out from the infected plant tissues are cut, put in fresh Potato Dextrose Agar (PDA) plates and maintained in PDA slants at 4°C.

### Morphological and cultural characterization

For studying morphological and cultural characterization the isolates were inoculated on PDA medium. The 20 ml medium was poured into each 90 mm petri plate and

inoculated with 5 day old inoculum of 5 mm disc by placing in centre of the Petri dish. Three replications for each isolate were maintained. The inoculated plates were incubated at 27 ±1°C. Mycelial and sclerotial parameters were recorded for each isolate.

### Pathogenic variability

Plants of the susceptible cultivar (TN-1) were inoculated at tillering stage with Typha bits colonized with *R. solani*, and were maintained in the glass house at 75-90 % relative humidity. The disease incidence and disease severity were recorded 7 days after inoculation, by measuring number of tillers affected, plant height and lesion height. Three replications for each isolates were maintained.

### DNA extraction and purification

Fungal DNA was extracted following the method of George *et al.*, (1998)

### RAPD analysis

Initially screened 25 RAPD RBa primers of which 10 primers yielded scorable and reproducible banding patterns were selected for further study. The reaction mixture composition for the polymerase chain reaction was 25 µl, containing 2.0 µl 10x Taq Buffer A, 1.0 µl 2.5 mM dNTPs, 1.0 µl Primer, 1.0 µl MgCl<sub>2</sub> and 0.3 µl Taq polymerase (all from Bangalore Genei, India). Then, 20 µl of master mix was added to another tube containing 1.5 µl of template DNA and a spin was given. The thermo cycling profile consisted of 1 cycle of initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 1 min (denaturation), 36°C for 1 min (primer annealing), 72°C for 2 min (extension), followed by a final extension at 72°C for 5 min. The amplified DNA samples were electrophoresed on 2.0 % agarose gel in 1x TAE buffer stained with ethidium bromide

along with 1kb DNA ladder and visualized under Gel Documentation System (GDS) (AlphaImager, USA).

### ISSR analysis

Fifty ISSR primers obtained from University of British Columbia website used for the study and found that 10 primers yielded scorable and reproducible banding patterns. These were selected for studying genetic variability. Amplification reactions were performed in a 25 µl volume containing 2.0 µl 10 x Taq Buffer A, 1.0 µl 2.5 mM dNTPs, 1.0 µl primer, 1.0 µl MgCl<sub>2</sub>, 0.3 µl Taq polymerase (3u) (from Bangalore Genei) and 14.0 µl of Milli-Q Water. The optimized PCR analysis was performed using a Veriti™ 96 gradient thermal cycler (Applied Biosystems, CA, USA) with the following amplification conditions: 1 cycle of initial denaturation at 95°C for 5 min followed by 30 cycles of 94°C for 1 min (denaturation), 36°C for 1 min (primer annealing), 72°C for 2 min (extension), followed by a final extension at 72°C for 5 min. The amplified DNA samples were electrophoresed on 2.0 % agarose gel in 1x TAE buffer stained with ethidium bromide along with 1kb DNA ladder and visualized under Gel Documentation System.

## Results and Discussion

### Morphological and cultural characterization

All the 20 isolates were grouped based on mycelial and sclerotial characters as per Lal *et al.*, (2014) and Upadhyay *et al.*, (2013). Morphological studies of the isolates showed wide variability in angle mycelial growth, mycelial width and distance between two septation. The isolate RS-K-17 branched at maximum degrees of angle (96.15<sup>0</sup>) followed by RS-K-12(93.84<sup>0</sup>), RS-K-1(93.74<sup>0</sup>) and RS-K-9(93.49<sup>0</sup>) (Table 1 and Fig. 1). Most of

isolates branched at nearly 90<sup>0</sup>. It was an obvious observation for the mycelial branching at right angles as a known feature of *R. solani* (Sneh *et al.*, 1991). Lal *et al.*, (2014) described that 25 isolates of *R. solani* and all the isolates exhibited typical hyphal branching at right angle. The perusal of data presented in Table 1 showed that the hyphal width of all the twenty isolates varied from 1.80 (RS-K-20) to 9.43 µm (RS-K-4). The maximum distance between the septation was observed in the isolate RS-K-11(351.92 µm) and isolate RS-K-18 showed minimum septation distance (15.37 µm). It was an obvious observation for the mycelium branching out at right angles, hyphal width 1.80 to 9.43 µm and distance between two septation were visualized under light microscopy and these were the characters of immense taxonomical importance which were described by the previous workers Duggar (1915), Matsumoto (1921), Singh *et al.*, (2014) and Moni *et al.*, (2016).

### Cultural variability

The details pertaining to cultural variability were presented in the Table 2. The colony color varied from Ivory to pale brown. Based on reverse surface color on the petri-plate 20 *R. solani* isolates were classified into three categories *viz.*, Ivory, sand yellow and olive green. Zhang *et al.*, (1995) allocated turf grass isolates of *R. solani* to different AG groups based on colony pigmentation. Similarly, Lal *et al.*, (2014) categorized *R. solani* isolates causing Sheath blight disease into five groups based on colony color. Distinct differences were observed in the colony appearance. The colony texture varied from highly dense texture *i.e.*, fluffy, flat plain and slightly fluffy mycelial growth. Distinct differences were observed in the colony appearance and the isolates were categorized into different groups based on texture and abundance of mycelium. Based on growth rate all the 20 isolates were categorized mainly into three groups *viz.*, slow

growing (1.8-2.0 mm/h), medium growing (2.0-2.2 mm/h) and fast growing ( $\geq 2.2$  mm/h). Similar observations had been made by Toda *et al.*, (1999) who divided *R. solani* AG-D isolates into two subgroups AG-D (I) and AG-D (II), based on the results of cultural characteristics. Guleria *et al.*, (2007) used cultural characters for differentiating the *R.*

*solani* isolates from rice. Thind and Aggarwal (2008), Khodaryari *et al.*, (2009) and Guleria *et al.*, (2007) stated that the *R. solani* isolates from rice were fast growing with  $>20$  mm mycelial growth rate per day indicating their fast growing nature. Rapid growth rate among *R. solani* isolates have also been reported by Lal *et al.*, (2014) and Upadhyay *et al.*, (2013).

**Table.1** Morphological characters of *Rizhoctonia solani* isolates from Karnataka

S. No	Isolates	Mycelial width (in $\mu\text{m}$ )	Angle of branching ( $^{\circ}$ )	Distance between septation (in $\mu\text{m}$ )
1	RS-K-01	5.48 <sup>cd</sup>	93.74	105.64 <sup>e</sup>
2	RS-K-02	8.96 <sup>ab</sup>	74.09 <sup>a</sup>	080.86 <sup>f</sup>
3	RS-K-03	8.80 <sup>ab</sup>	78.02 <sup>a</sup>	109.43 <sup>de</sup>
4	RS-K-04	9.43 <sup>a</sup>	68.30 <sup>a</sup>	229.01 <sup>b</sup>
5	RS-K-05	8.06 <sup>b</sup>	87.89 <sup>b</sup>	119.97 <sup>d</sup>
6	RS-K-06	8.09 <sup>b</sup>	86.30 <sup>b</sup>	169.45 <sup>c</sup>
7	RS-K-07	9.30 <sup>a</sup>	90.65 <sup>b</sup>	078.58 <sup>f</sup>
8	RS-K-08	8.48 <sup>ab</sup>	80.16 <sup>a</sup>	057.16 <sup>gh</sup>
9	RS-K-09	2.41 <sup>e</sup>	93.49 <sup>b</sup>	187.08 <sup>c</sup>
10	RS-K-10	2.25 <sup>e</sup>	86.84 <sup>b</sup>	056.41 <sup>gh</sup>
11	RS-K-11	8.63 <sup>ab</sup>	74.53 <sup>a</sup>	351.92 <sup>a</sup>
12	RS-K-12	8.94 <sup>ab</sup>	93.84 <sup>b</sup>	045.35 <sup>i</sup>
13	RS-K-13	9.02 <sup>ab</sup>	84.42 <sup>b</sup>	107.31 <sup>de</sup>
14	RS-K-14	2.38 <sup>e</sup>	78.62 <sup>a</sup>	062.51 <sup>g</sup>
15	RS-K-15	6.15 <sup>c</sup>	91.54 <sup>b</sup>	252.23 <sup>b</sup>
16	RS-K-16	5.03 <sup>d</sup>	89.72 <sup>b</sup>	052.03 <sup>hi</sup>
17	RS-K-17	1.92 <sup>e</sup>	96.15 <sup>b</sup>	057.03 <sup>gh</sup>
18	RS-K-18	2.44 <sup>e</sup>	86.87 <sup>b</sup>	015.37 <sup>j</sup>
19	RS-K-19	2.41 <sup>e</sup>	83.99 <sup>b</sup>	164.44 <sup>c</sup>
20	RS-K-20	1.80 <sup>e</sup>	87.76 <sup>b</sup>	064.69 <sup>g</sup>
	C.D. ( $P=0.05$ )	1.05	21.56	0.06
	C.V (%)	10.56	15.18	1.97

**Table.2** Cultural characteristics of *Rhizoctonia solani* isolates collected from rice growing areas of Karnataka

Isolates	Surface color of the culture plate	Reverse color of the culture plate	Mycelial color	Arrangement of sclerotia	Texture	Honey dew secretion
RS-K-1	Ivory	Sand yellow	Cream	Concentric rings	Fluffy	-
RS-K-2	Olive grey	Sand Yellow	White	Lower and peripheral ring	Flat plain	+
RS-K-3	Sand yellow	Sand yellow	Oyster white	Grouped at centre	Flat plain	-
RS-K-4	Sand yellow	Sand Yellow	Cream	Scattered grouping	Slightly fluffy	+
RS-K-5	Sand yellow	Sand yellow	Cream	Grouped at centre and peripheral ring	Fluffy	+
RS-K-6	Pale brown	Ivory	White	Lower ring	Slightly fluffy	+
RS-K-7	Sand yellow	Sand Yellow	Signal white	Lower ring	Flat plain	-
RS-K-8	Sand yellow	Olive Grey	Light ivory	Scattered grouping	Slightly fluffy	-
RS-K-9	Pale brown	Ivory	Cream	Grouped at centre	Slightly fluffy	+
RS-K-10	Pale brown	Sand Yellow	Cream white	Scattered grouping	Fluffy	+
RS-K-11	Olive grey	Olive Grey	Cream	Grouped at centre	Slightly fluffy	-
RS-K-12	Sand Yellow	Sand yellow	White	Lower ring	Slightly fluffy	-
RS-K-13	Ivory	Ivory	Oyster white	Middle and peripheral ring	Slightly fluffy	-
RS-K-14	Pale brown	Ivory	White	Scattered grouping	Flat plain	-
RS-K-15	Sand Yellow	Olive Grey	White	Middle ring	Flat plain	-
RS-K-16	Ivory	Ivory	White	Grouped at centre, peripheral ring	Slightly fluffy	+
RS-K-17	Ivory	Ivory	Oyster white	Grouped at centre	Slightly fluffy	+
RS-K-18	Sand Yellow	Sand yellow	Light ivory	Middle ring	Flat plain	+
RS-K-19	Sand yellow	Sand Yellow	Cream	Grouped at centre, Scattered grouping	Slightly fluffy	-
RS-K-20	Ivory	Sand Yellow	Sand Yellow	Grouped at centre	Slightly fluffy	-

**Table.4** Pathological variation among *R. solani* isolates collected from major rice growing areas of Karnataka

Isolates	No of tillers	Infected tillers	First lesion from the Base	No of lesion		Distance b/w two lesion (cm)	Area of Lesion (Cm <sup>2</sup> )	Lesion structure	Lesion color	Plant height	Lesion height	Mean Disease score	Relative Lesion height (RLH)
				Sheath	Leaf								
RS-K-1	20	19	3	8	0	0.3	1.05	Elliptical, Circular, Amorphous,	Grey	66.0	18.0	4	27.70
RS-K-2	20	20	7.1	6	2	0.3	2.50	Elliptical, Amorphous, Elongated	Light greenish,	59.0	18.7	5	31.66
RS-K-3	20	20	4.5	4	0	0.2	0.72	Elliptical	Grey	63.0	20.7	5	32.83
RS-K-4	20	20	2.9	5	0	0.2	1.76	Elliptical, Elongated		61.0	20.7	5	32.30
RS-K-5	20	18	2.5	10	0	0.3	3.15	Elliptical, Elongated	Grey	74.7	23.7	4	32.00
RS-K-6	20	11	1.5	3	0	2	1.08	Elongated	Brown, Grey	72.0	15.0	3	20.83
RS-K-7	20	12	2	3	0	0.2	0.40	Elongated	Light greenish/ Brown	70.0	18.7	4	26.66
RS-K-8	20	20	1.2	13	0	0.3	1.20	Elliptical, Elongated	Light greenish/ Grey	65.0	21.0	4	29.03
RS-K-9	20	20	1.6	7	0	0.3	1.00	Elliptical, Elongated	Light greenish/ Grey	66.7	21.0	5	31.46
RS-K-10	20	13	1.5	1	0	0	0.90	Elongated	Brown	64.7	14.0	3	21.63
RS-K-11	20	16	5.2	8	0	0.3	0.90	Elliptical, Elongated	Light greenish,	61.7	16.7	5	27.03
RS-K-12	20	20	3.8	4	0	0.6	1.89	Elliptical, circular	Grey	61.0	18.7	5	33.86
RS-K-13	20	20	6.2	5	0	0.0	1.52	Elongated	Grey	62.0	18.0	4	29.03
RS-K-14	15	17	1.5	7	0	0.4	1.10	Elliptical	Light greenish, Grey	68.0	21.7	6	31.86
RS-K-15	20	18	2.8	6	0	0.2	1.40	Elliptical	brown	66.0	21.7	5	33.86
RS-K-16	20	16	1	1	0	0.1	0.90	Elongated	Brown	61.0	10.7	3	17.26
RS-K-17	20	20	13.5	5	0	0.3	0.39	Elliptical, Elongated	Light greenish, brown	66.7	18.7	3	28.03
RS-K-18	20	20	3.8	9	0	0.3	0.65	Elongated	Light greenish,	63.0	18.7	5	29.63
RS-K-19	20	11	1.8	3	0	2.1	1.76	Elliptical, Elongated	Light greenish, Grey	70.0	14.0	3	20.00
RS-K-20	20	17	14	5	1	0.4	0.15	Elliptical, Amorphous, Circular	Light greenish,	65.0	21.0	5	30.63
			CD (P=0.05)										5.105
			CV (%)										10.865

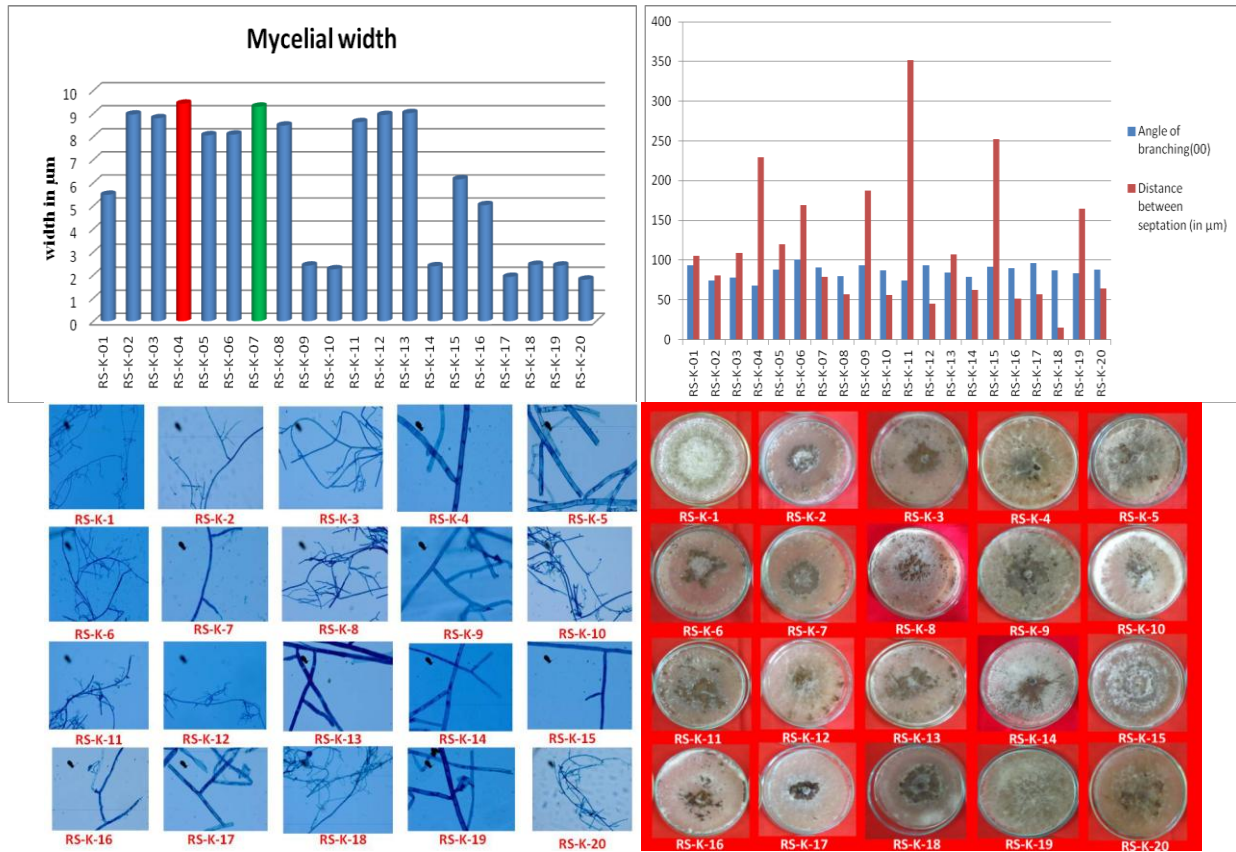
**Table.3** Pathological variability of *Rhizoctonia solani* isolates collected from rice growing areas of Karnataka

Sl. No.	Isolates	RLH	Disease incidence
1	RS-K-1	27.70	96.67 (80.43)
2	RS-K-2	31.66	100.00 (87.13)
3	RS-K-3	32.83	100.00 (87.13)
4	RS-K-4	32.30	100.00 (87.13)
5	RS-K-5	32.00	90.00 (74.04)
6	RS-K-6	20.83	83.33 (65.95)
7	RS-K-7	26.66	58.33 (49.80)
8	RS-K-8	29.03	100.00 (87.13)
9	RS-K-9	31.46	100.00 (87.13)
10	RS-K-10	21.63	65.00 (53.72)
11	RS-K-11	27.03	78.33 (62.29)
12	RS-K-12	33.86	100.00 (87.13)
13	RS-K-13	29.03	100.00 (87.13)
14	RS-K-14	31.86	55.00 (47.87)
15	RS-K-15	33.86	90.00 (71.56)
16	RS-K-16	17.26	80.00 (63.93)
17	RS-K-17	28.03	98.33 (83.78)
18	RS-K-18	29.63	100.00 (87.13)
19	RS-K-19	20.00	56.67 (48.83)
20	RS-K-20	30.63	85.00 (67.40)
C.D.		5.105	6.37
C.V.		10.865	5.26

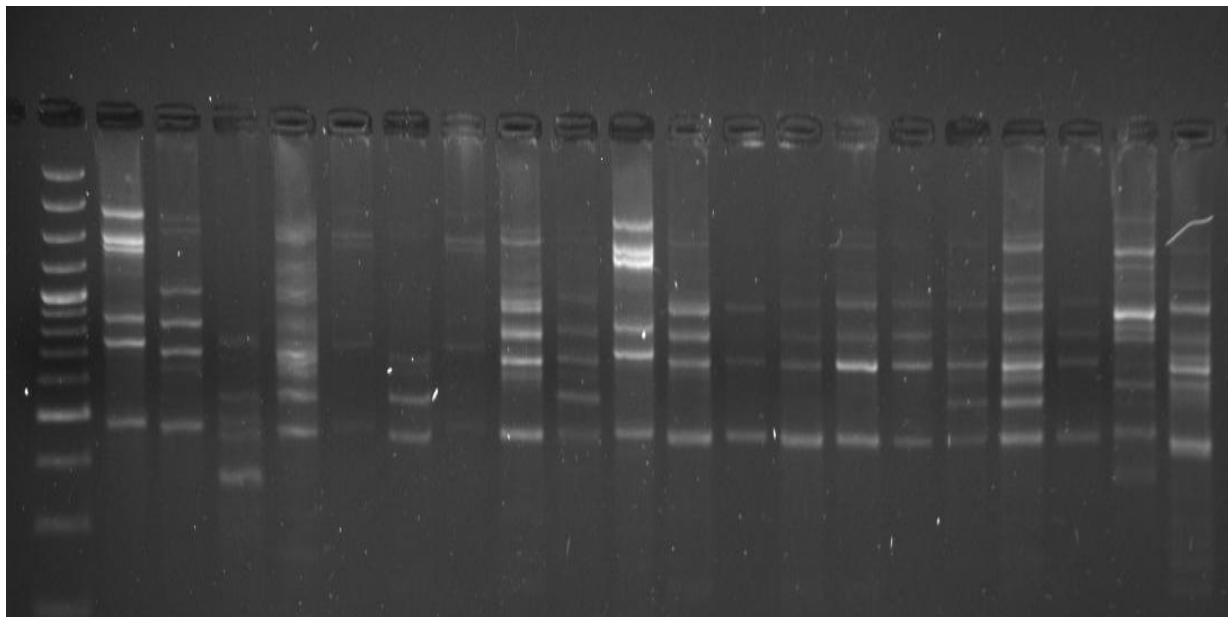
**Table.5** Details of the primers, polymorphism and banding patterns of 20 isolates of *Rhizoctonia solani* by 10 RAPD primers

RAPD Primers (RBa)	No. of loci	No. of polymorphic loci	Polymorphism %	PIC	Mean Genetic similarity
2	12	12	100.00	0.8778	0.31
3	7	7	100.00	0.8244	0.29
5	12	12	100.00	0.8733	0.36
6	8	8	100.00	0.8362	0.38
8	8	8	100.00	0.8520	0.57
9	16	16	100.00	0.8668	0.29
13	10	10	100.00	0.8519	0.33
20	10	10	100.00	0.8633	0.51
22	11	11	100.00	0.8529	0.51
23	8	8	100.00	0.8318	0.32

**Fig.1** Mycelial width and angle of branching of isolates collected from major rice growing areas of Karnataka

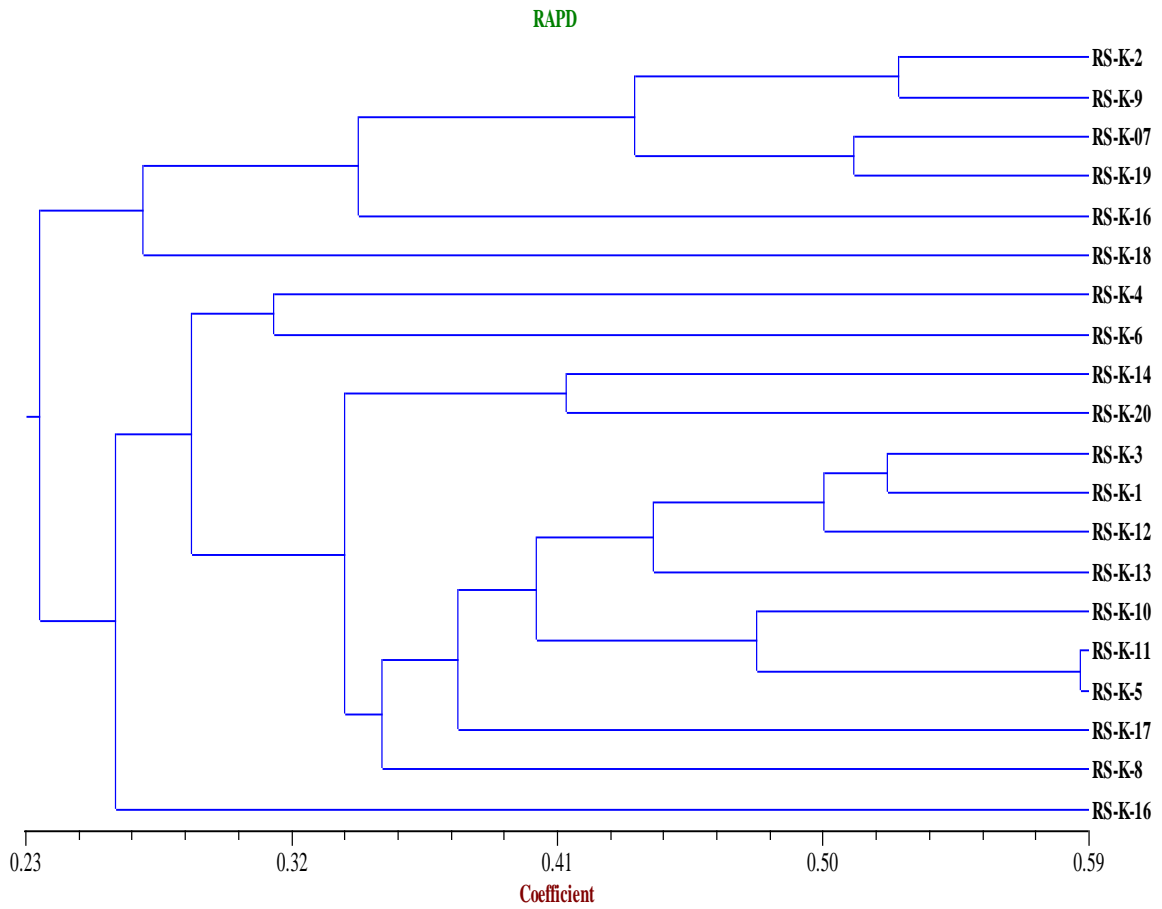


**Fig.2** Electrophoretic banding pattern of 20 *R. solani* isolates of using RAPD primers, RBa-20

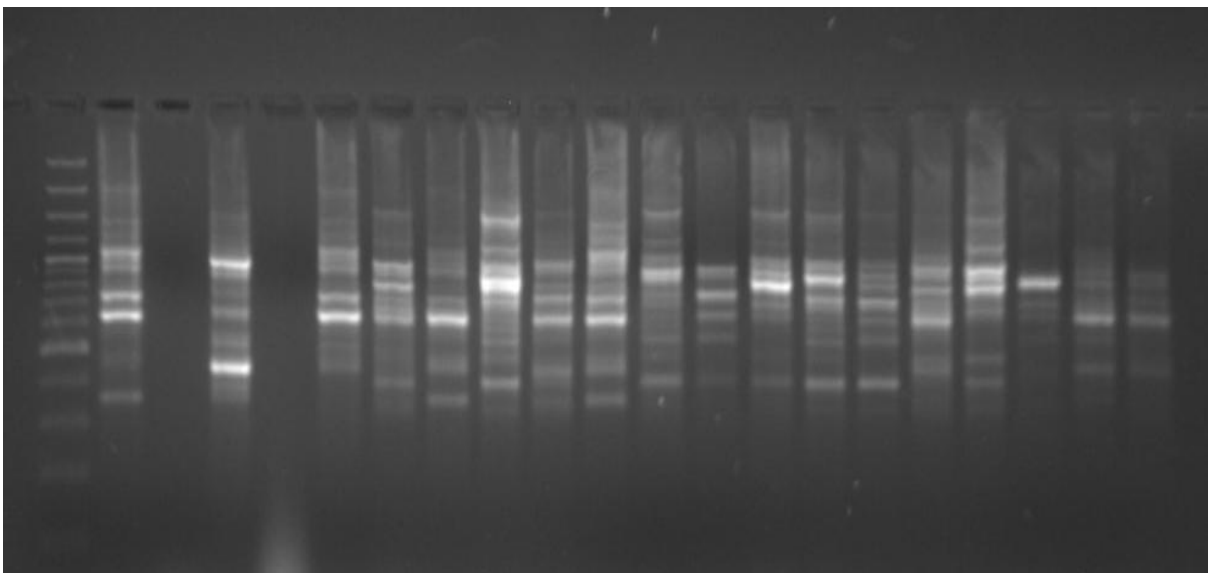




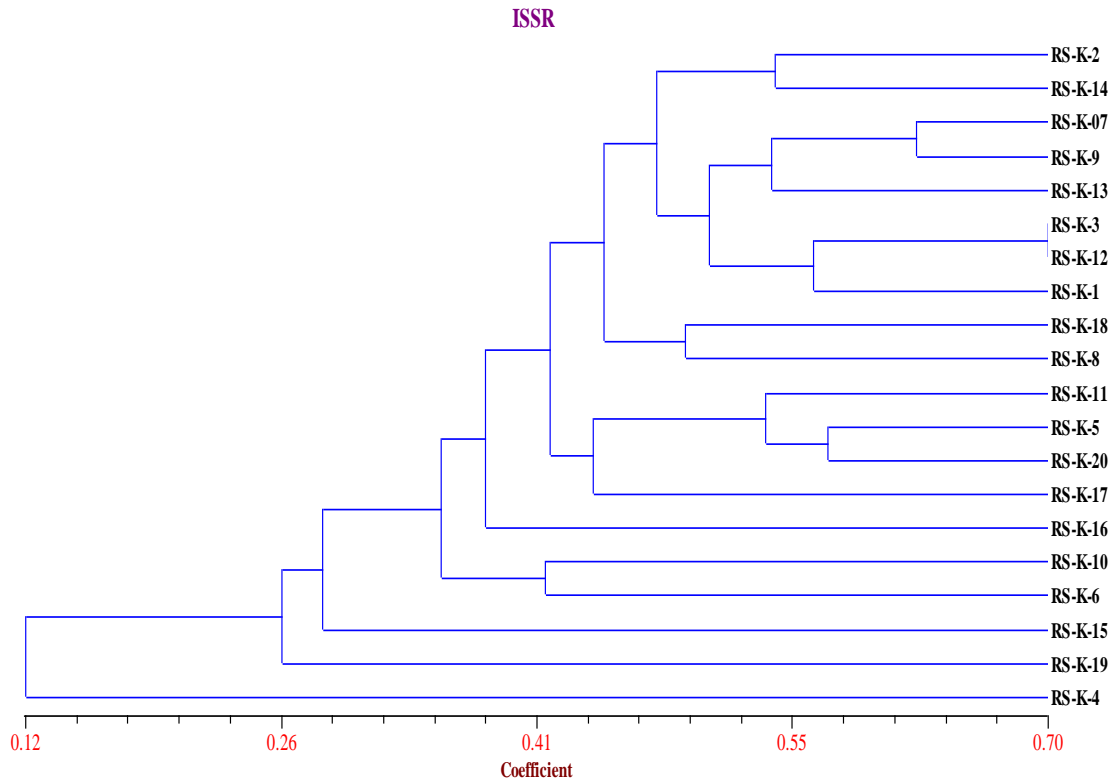
**Fig.3** UPGMA dendrogram showing clustering of 20 isolates of *R. solani* using pooled RAPD



**Fig.4** Electrophoretic banding pattern of 20 *R. solani* isolates of using ISSR primers, UBC-808



**Fig.5** UPGMA dendrogram showing clustering of 20 isolates of *R. solani* using pooled ISSR



Based on pattern of sclerotial production 20 *R. solani* isolates were grouped into five categories viz., sclerotia grouped at centre(7), lower ring (3), middle ring (2), peripheral ring (4) and scattered (4). Among 20 *R. solani* isolates nine isolates secreted honey dew and others did not. However maximum number of sclerotia observed was 618 (RS-K-1), while minimum number of sclerotia was 67 (RS-K-5). The sclerotial weight of 20 isolates ranged from 8.75 to 18.50 mg. Such type of categorization based on the pattern of formation and arrangement among rice *R. solani* isolates was done by Upadhyay *et al.*, (2013) Thakur *et al.*, (1992), Guleria *et al.*, (2007) Singh *et al.*, (2014) and Kumar *et al.*, (2008).

#### **Pathological variability**

Pathological variability of 20 *R. solani* isolates was studied on susceptible cv. TN-1

and found that isolates took 2-5 days to exhibit the typical sheath blight symptoms. And the size of the lesion ranged from 0.15-3.15 cm<sup>2</sup>, most of the isolates produced either elliptical or elongated lesions (Table 4). The disease severity varied from 17.26-33.86 %, among the isolates. All the twenty isolates were classified highly virulent (>50 % DI) based on per cent disease incidence (Table 3). These results were in accordance with the findings of Swain *et al.*, (2005) Nandi and Chakrabarthy (1984) Basu and Gupta (1992) Xiao *et al.*, (2008)

#### **Molecular variability**

Genetic diversity of *R. solani* isolates from different locations using 10 RAPD and ISSR primers showed good polymorphism at DNA level and cluster analysis of RAPD data grouped the isolates on the basis of their origin with few exceptions. A total of 102

amplicons were obtained from twenty *R. solani* isolates with 10 RAPD primers of which 100 % were polymorphic (Table 5). Cluster analysis of sheath blight isolates revealed the average pair-wise similarities in the range of 0.29-0.57 thus suggesting large variations among the isolates. In case of ISSR primers a total of 111 were obtained with 10 ISSR primers of which 95.92 % were polymorphic. Cluster analysis revealed that 20 isolates grouped into two major clusters at 35 % genetic similarity coefficient (Fig. 2-5). Similarity coefficient ranged from 0.30-0.50 thus suggests good variations among the isolates. In the past, several studies were conducted for assessing molecular diversity in *R. solani* were conducted using RAPD based fingerprinting Sharma *et al.*, (2005), Sundravadana *et al.*, (2011) and Banerjee *et al.*, (2012). Similar results were reported by Zhou *et al.*, (2002), Yugander *et al.*, (2015) who analysed genetic variability using ISSR primers.

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**How to cite this article:**

Manjunatha, O., B. Vidya Sagar, V. Prakasam and Narendra Reddy, C.N. 2018. Variability Studies on Sheath Blight of Rice in Karnataka, India. *Int.J.Curr.Microbiol.App.Sci*. 7(10): 724-736. doi: <https://doi.org/10.20546/ijcmas.2018.710.080>