

## Virulence spectrum among *Rhizoctoniasolanif. sp. sasakii* isolates from multi-geographical locations of India

### ABSTRACT

*Rhizoctonia solani* f. sp. *sasakii* (RSS) is a highly devastating soil-borne fungal pathogen inciting banded leaf and sheath blight (BLSB) disease in maize. In India, annually one percent of the total maize yield is reduced by BLSB. Due to continuous occurrence and wide spread of disease, the present experiment was conducted ~~at screen house condition of CCS Haryana Agricultural University, Regional Research Station Karnal~~ to assess the pathogenic variability among different isolates obtained from multi-geographical locations of India. Pathogenic behaviour of each isolates was predicted through the comparable study of their incubation period, average disease score and disease intensity on the three maize hybrids (Normal maize- HM 8, QPM maize- QPM 9 and sweet corn- HSC I). The incubation period of all the *R. solani* isolates ranged from 3 to 7 days with average disease score ranged from 2.1 to 7.1, depending upon the host and isolate interaction after artificial inoculation. Disease intensity of all the ~~R. solani~~ RSS isolates on maize hybrids varied from 8.1 to 52.1% ~~per cent and while~~ isolates RS 29 (New Delhi) and RS 1 (Karnal) were found highly virulent, in comparison to other forty nine isolates of ~~R. solani~~ RSS. As these isolates showed susceptible reaction on maize hybrids with average disease score ranged from 6.2 to 7.1, whereas the isolates RS 30 (Dholi) and RS 31 (Dhaura Kuan) were recorded as least virulent, showed resistant disease reaction with average disease score ranged from 1.5 to 2.4.

**Keywords:** Maize, BLSB, *Rhizoctonia solani* f. sp. *sasakii*, virulence spectrum, pathogenic variability

### 1. INTRODUCTION

The pathogen *Rhizoctonia solani* f. sp. *sasakii* (Kuhn) Exner, (teleomorph= *Thanatephorus cucumeris* Frank (Donk) belonging to kingdom Fungi, phylum Basidiomycota, class Agaricomycetes, order Cantharellales, family Ceratobasidiaceae and genus *Rhizoctonia* which was firstly demonstrated by De Candolle in 1815. It is a genetically diverse and destructive fungus with a wide host range causing economically important diseases in tropical, sub-tropical and temperate regions [2]. The variability in the pathogen due to hyphal anastomosis responsible for its high virulence, aggressiveness making it complicated in the host range and in screening of host in resistance program. *R. solani* f. sp. *sasakii* is a versatile pathogen with a wide host range causes many diseases such as damping-off, root rot, seed or cob decay and stem canker and capable to adapt itself in to diverse agro-climatic ecosystem [10]. The *R. solani* is a versatile, necrotrophic pathogen can persistent in soil and act as destructive pathogen. It can infects a wide range of host plants and also have highly competitive saprophytic ability [14, 15, 175].

In maize the pathogen ~~Rhizoctonia~~ *R. solani* f. sp. *sasakii* incited the banded leaf and sheath blight

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disease of maize. In India, banded leaf and sheath blight disease of maize was firstly reported as a minor disease [12]. Later, it gained epidemic dimensions across Himalayas region and foot hills of Mandi district of Himachal Pradesh [21]. Presently, the disease is considered as a major constraint not only in India and Sri Lanka but is attaining epidemic attributes in major maize growing countries such as Japan, Venezuela, Nigeria, Bhutan, Costa Rica, Ivory Coast, Sierra Leone, England, Nepal, Korea, Southern China, Philippines, Pakistan, Malaysia, Indonesia and Vietnam [16], [18]. Now, the banded leaf and sheath blight is known as highly destructive disease in Himachal Pradesh, Assam, Meghalaya, Uttar Pradesh, Bihar, Nagaland, Jammu Kashmir, Haryana, Uttarakhand, Punjab, Sikkim, Madhya Pradesh, Delhi, Rajasthan, Orissa, Andhra Pradesh and West Bengal [19], [1], [14]. In India, annually one percent of the total maize yield is reduced by BLSB, the losses in terms of grain yield may estimate to the range of 11-40 per cent, even to 100 per cent specially in Haryana due to continue rains in the months of July and August [6], [13], [17], [8], [4].

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## 2. MATERIALS AND METHODS

### 2.1 Collection and maintenance of infected samples

Banded leaf and sheath blight (BLSB) disease infected samples were collected from major maize growing regions of India during *kharif* 2019 and 2020 at various growth stages of crop. The infected leaf and sheath samples were brought to the laboratory and stored in paper bags for further isolation of the pathogen.

### 2.2 Isolation and purification of *R. solani* f. sp. *sasakii* isolates

Banded leaf and sheath infected samples collected from multi geographical locations of India were washed in running tap water, approximate 1 cm size diseased portion of the infected sample of leaf and sheath along with healthy portion were cut with a sterilized scalpel blade. The cut samples were surface sterilized in 1% sodium hypochlorite for 1 or 2 minutes followed by three time proper rinsing with sterilized distilled water and dried on blotter paper. Surface sterilized leaf bits were transferred to 2 per cent water agar medium in Petri plates supplemented with streptomycin sulphate for prevention of bacterial contamination and incubated for 48 hours at 25 ±2°C. Fine mycelium growth was observed from edge of the infected bits after two days of incubation. To obtain pure culture a hyphal tip was transferred to fresh PDA plate. The *R. solani* RSS isolates collected from different locations were designated as RS 1 to RS 51 as shown in Table 2. The pure cultures were preserved on PDA slants in the refrigerator at 4°C for the further experiments.

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### 2.3 Identification and maintenance of the *R. solani* f. sp. *sasakii* isolates

The isolates of *R. solani* RSS were identified based on cultural (colony colour, texture and appearance), sclerotial and morphological characters (branching at right angle and constriction of branch). Pure cultures of fifty one *R. solani* isolates collected from major maize growing districts of India were

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identified and grown on PDA slants. These slants were sub cultures on new slants at regular time intervals and stored at 4°C.

**Table 1: Isolates of *R. solani* collected from different geographical locations**

Sr. No.	Designation	Place of isolates	State
1	RS 1	Uchani (Karnal)	Haryana
2	RS 2	Kuchpura (Karnal)	Haryana
3	RS 3	Indri (Karnal)	Haryana
4	RS 4	Nissing (Karnal)	Haryana
5	RS 5	Saha (Ambala)	Haryana
6	RS 6	Saphera (Ambala)	Haryana
7	RS 7	Jaloli (Ambala)	Haryana
8	RS 8	Behlon (Panchkula)	Haryana
9	RS 9	Tikkar Taal, Morni (Panchkula)	Haryana
10	RS 10	Bariya, Kalka (Panchkula)	Haryana
11	RS 11	Kidarpur, Kalka (Panchkula)	Haryana
12	RS 12	Rapouli, Mustafabad (Yamunanagar)	Haryana
13	RS 13	Basant Pura, Radaur (Yamunanagar)	Haryana
14	RS 14	Ladwa (Kurukshetra)	Haryana
15	RS 15	Shahbad, Markanda (Kurukshetra)	Haryana
16	RS 16	Behlolpur (Panipat)	Haryana
17	RS 17	Aterna (Sonipat)	Haryana
18	RS 18	Manouli (Sonipat)	Haryana
19	RS 19	Bhirdana (Fatehabad)	Haryana
20	RS 20	Darbi (Sirsa)	Haryana
21	RS 21	Hansi (Hisar)	Haryana
22	RS 22	Dera Bassi	Punjab
23	RS 23	Ludhiana	Punjab
24	RS 24	Hoshiarpur	Punjab
25	RS 25	Udiapur	Rajasthan
26	RS 26	Pantnagar	Uttarakhand
27	RS 27	Almora	Uttarakhand
28	RS 28	New Delhi	New Delhi
29	RS 29	New Delhi	New Delhi
30	RS 30	Dholi	Bihar
31	RS 31	Dhaura Kuan	Himachal Pradesh
32	RS 32	Varanasi	Uttar Pradesh
33	RS 33	Maunath Bhanjan	Uttar Pradesh
34	RS 34	Mirzapur	Uttar Pradesh
35	RS 35	Barapani	Meghalaya
36	RS 36	Kalyani	West Bengal
37	RS 37	Ranchi	Jharkhand
38	RS 38	Hyderabad	Telangana
39	RS 39	Dharwad	Karnataka
40	RS 40	Coimbatore	Tamil Nadu
41	RS 41	Karnal	Haryana
42	RS 42	Kaul	Haryana
43	RS 43	Kaul	Haryana
44	RS 44	Fatehabad	Haryana
45	RS 45	Hisar	Haryana
46	RS 46	Sirsa	Haryana
47	RS 47	Karnal	Haryana
48	RS 48	Rewari	Haryana

49	RS 49	Hisar	Haryana
50	RS 50	Hisar	Haryana
51	RS 51	Hisar	Haryana

## 2.4 Pathogenic variability among different isolates of *R. solani* under screen house

### 2.4.1 Raising of crop in screen house

The pathogenic variability experiment was conducted at screen house condition of CCS Haryana Agricultural University, Regional Research Station Karnal. For each isolate, three hybrids (HM 8, QPM 9 and HSC I) were sown individually in separate pots with the four replications.

### 2.4.2 Preparation of mass culture and artificial inoculation

The mass culture of each isolate of *R. solani* on barley grains was prepared. Barley grains were soaked in water for 24 hours and dispensed 40 g seeds in 250 ml Erlenmeyer flask after removing excess of water and autoclaved at 121.6°C at 15 lbs for 20 minutes. Each flask was inoculated with 5 mm mycelium disc of *R. solani* isolates, derived from the actively growing cultures on the PDA plates and incubated at 25 ± 2°C in BOD incubator for ten days. The fully grown fungal mycelium on barley grains were dispensed out from flask on a paper for drying at 15°C. Pathogenic variability of fifty one isolates (RS 1 to RS 51) was determined by inoculating on 30-35 days old plants of each hybrid using the leaf sheath inoculation method (Ahuja and Payak, 1978). Fungal mycelium coated grain were inserted between stalk at second or third internodes level from soil for inoculation. Water was sprayed on each plant after inoculation to maintain high relative humidity and soil moisture for disease development and progression. The final observation on incubation period, average disease score and disease intensity were recorded for each isolate on maize hybrids 15 days after inoculation.

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### 2.4.3 Observations recorded

#### 2.4.3.1 Incubation period

For each isolate on inoculated hybrid, the incubation period is defined as the time (days) from inoculation to the emergence of the first chlorotic lesions. The examination of first lesion appeared on plant from first day inoculation to tenth day of inoculation for assessment of incubation period.

#### 2.4.3.2 Average disease rating

The average disease rating was given from base of plant to the tip of top most lesions on stalk and using rating scale of 1-9 (Table 2) [3] as follow.

**Table 2: Disease rating scale for assessment of banded leaf and sheath blight of maize**

Rating scale	Disease reaction	PDI (%)
1.0-3.0	Resistant (R)	0.00-33.33%
4.0-5.0	Moderately resistant (MR)	44.44-55.55%
6.0-7.0	Moderately susceptible (MS)	66.66-77.77%

### 2.4.3.3 Disease intensity

The disease intensity was calculated using formula [7] for each isolate on each hybrid 15 days after inoculation following 1-9 rating scale [3].

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of plant assessed} \times \text{maximum rating}} \times 100$$

## 3. RESULTS AND DISCUSSION

### 3.1 Characterization of *Rhizoctonia solani* f. sp. *sasakii* isolates

A total of ~~fifty one~~ 51 *R. solani* f. sp. *sasakii* isolates were collected from major maize growing regions of India during *kharif* 2019 and 2020. Isolates were designated as RS 1 to RS 51 and identified with respect to their cultural, morphological and molecular characters.

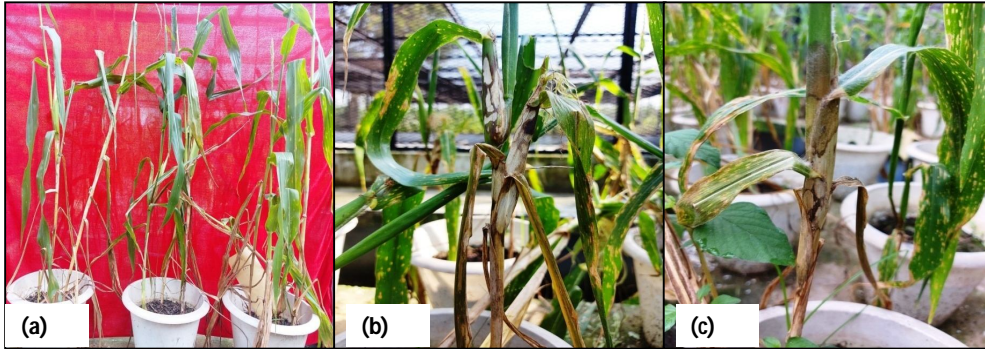
### 3.2 Pathogenic variability among different isolates of *R. solani* f. sp. *sasakii*

Pathogenic behavior of fifty one *R. solani* isolates was predicted through the comparable study of their incubation period, average disease score and disease intensity on the three maize hybrids (Normal maize- HM 8, QPM maize- QPM 9 and sweet corn- HSC I). The significant variations were observed in the incubation periods of fifty one *R. solani* isolates on the maize hybrids as presented in Table 3 and Figure 1. The incubation period of all the *R. solani* isolates ranged from 3 to 7 days depending upon the host and isolate interaction after artificial inoculation. The shortest incubation period of eleven *R. solani* isolates (RS 1, RS 2, RS 3, RS 7, RS 8, RS 10, RS 16, RS 23, RS 29, RS 37 and RS 44) were observed in hybrid HM 8 up to 4 days, whereas the longest incubation period of 7 days was observed in four isolates (RS 17, RS 19, RS 25 and RS 33). In case of hybrid QPM 9, the shortest of incubation period 3 days were recorded in seven isolates (RS 2, RS 8, RS 10, RS 16, RS 23, RS 29 and RS 44), while the longest incubation period of 7 days was found in four isolates (RS 13, RS 17, RS 25 and RS 34). Similarly, in the sweet corn hybrid HSC I, the shortest incubation period 4 days observed in eleven isolates *viz.*, RS 1, RS 2, RS 3, RS 7, RS 8, RS 10, RS 16, RS 23, RS 29, RS 37 and RS 44, whereas longest incubation period of 7 days recorded in four isolates (RS 17, RS 19, RS 25 and RS 33).

**Table 3: Pathogenic variability among different isolates of *R. solanif. sp. sasakii***

Isolates	HM 8			QPM 9			HSC I		
	Incubation period (days)	Average rating scale	Disease intensity (%)	Incubation period (days)	Average rating scale	Disease intensity (%)	Incubation period (days)	Average rating scale	Disease intensity (%)
RS 1	4	6.6	46.4	4	7	51.4	4	6.2	48.9
RS 2	4	6.3	42.5	3	6.7	47.5	4	5.9	44
RS 3	4	5.4	34.3	4	5.6	37.3	4	5	33.8
RS 4	6	3.7	15.4	6	3.9	18.4	6	3.3	14.9
RS 5	5	4.2	39.5	4	4.4	42.5	5	3.8	38.5
RS 6	5	4.6	35.7	6	4.8	40.7	5	4.2	36.7
RS 7	4	2.2	11.5	4	2.6	16.5	4	1.8	12.5
RS 8	4	5.7	42.2	3	6.1	47.2	4	5.7	43.2
RS 9	6	3.6	19.7	6	4	24.7	6	3.6	21.2
RS 10	4	6	44.7	3	6.4	49.7	4	6	46.2
RS 11	5	4.7	32.5	4	5.1	38.5	5	4.7	36.5
RS 12	5	6.2	33.1	5	6.6	39.1	5	5.8	37.1
RS 13	6	2.6	13.2	7	3	19.2	6	2.2	17.2
RS 14	5	4.8	36.8	4	5.2	42.8	5	4.4	40.8
RS 15	5	5.3	35.4	5	5.7	41.4	5	4.9	39.4
RS 16	4	5.6	43.2	3	6	49.2	4	5.7	47.2
RS 17	7	3.1	16.5	7	3.5	22.5	7	3.2	20.5
RS 18	6	2.8	10.2	6	3.2	15.2	6	2.9	10.5
RS 19	7	2.3	9.6	6	2.7	14.6	7	2.4	9.9
RS 20	6	3.5	25.3	6	3.9	30.3	6	3.7	25.6
RS 21	6	3.7	27.4	4	4.1	32.4	6	3.9	27.7
RS 22	5	4.9	33.3	5	5.2	38.3	5	5	34.8
RS 23	4	6.4	47.3	3	6.7	49.3	4	6	45.8
RS 24	6	3.1	21.6	6	3.4	23.6	6	2.7	20.1
RS 25	7	2.3	12.7	7	2.6	14.7	7	1.8	11.2
RS 26	6	3.5	19.5	6	3.8	21.5	6	3	17.2
RS 27	6	3.7	20.1	6	4	22.1	6	3.2	17.8
RS 28	6	2.9	18.3	6	3.2	20.3	6	2.3	16
RS 29	4	6.8	48.2	3	7.1	52.1	4	6.2	49.1
RS 30	6	2.1	8.7	6	2.4	11.4	6	1.5	8.1
RS 31	6	2.1	8.9	6	2.4	11.7	6	1.6	8.8
RS 32	6	2.6	20.1	6	2.9	25.1	6	2.1	21.6
RS 33	7	3	28.5	6	3.3	33.5	7	2.5	30
RS 34	6	3.6	22.6	7	4	29.6	6	3.2	26.1

RS 35	6	3.1	25.5	6	3.5	32.5	6	2.7	29
RS 36	6	3.7	28.2	6	4.1	35.2	6	3.7	30
RS 37	4	6.1	43.8	4	6.5	52.8	4	6.1	42.6
RS 38	6	2.8	26.5	6	3.2	33.5	6	2.8	28.3
RS 39	5	5.1	36.9	4	5.5	41.9	5	4.7	36.7
RS 40	6	2.4	10.6	6	2.8	15.6	6	2	10.4
RS 41	5	5.3	39.1	4	5.7	44.1	5	4.9	38.9
RS 42	5	5.7	37.5	4	6.2	42.5	5	5.4	39
RS 43	6	2.7	26.4	6	3.2	31.4	6	2.4	27.9
RS 44	4	6.1	44	3	6.4	47.7	4	5.6	44.6
RS 45	6	3.4	21.1	6	3.7	22.1	6	3.1	19
RS 46	6	3.3	19.4	6	3.6	20.4	6	3	17.3
RS 47	5	3.4	14.3	4	3.9	15.3	5	3.3	12.2
RS 48	5	4.7	19.3	4	5.1	24.3	5	4.5	21.2
RS 49	5	3.2	13.4	5	3.6	17.4	5	2.8	13.9
RS 50	6	3	13.1	6	3.4	17.1	6	2.6	13.6
RS 51	5	5.3	37.5	5	5.7	41.5	5	4.9	38
RS 49	5	3.2	13.4	5	3.6	17.4	5	2.8	13.9
RS 50	6	3	13.1	6	3.4	17.1	6	2.6	13.6
RS 51	5	5.3	37.5	5	5.7	41.5	5	4.9	38



**Figure 1: Pathogenic variation in typical symptoms (a-c) among different isolates of *R. solani* on different maize hybrids under screen house conditions**

Greater extents of variation were found in the disease score of all the *R. solani* isolates on maize hybrids after 15 days of inoculation as presented in Table 3. The average disease score among all the *R. solani* isolates ranged from 2.1 to 7.1 depending upon the host and isolate interaction after artificial inoculation. Two isolates RS 29 and RS 1 were showed highest average disease score in all the maize hybrids. The isolate RS 29 from New Delhi was showed highest average disease score of 6.8 in maize hybrid HM 8, followed by RS 1 from Karnal showed 6.6 average disease score, whereas two isolates RS 30 from Dholi and RS 31 from Dhaula Kuan showed lowest average disease score of 2.1. In hybrid QPM 9, the highest average disease score 7.1 and 7.0 were observed in two isolates (RS 29 and RS 1), meanwhile the lowest average disease score 2.4 was recorded in two isolates RS 30 and RS 31. Similarly, in the sweet corn hybrid HSC I, the highest disease score 6.2 was recorded in two isolates (RS 29 and RS 1) and lowest 1.5 and 1.6 in RS 30 and RS 31 isolates.

The disease intensity of all the *R. solani* isolates on maize hybrids varied from 8.1 to 52.1 per cent (Table 3). The isolates RS 29 from New Delhi and RS 1 from Karnal were found highly virulent, in comparison to other forty nine isolates of *R. solani*. As these isolates showed susceptible reaction on maize hybrids with average disease score ranged from 6.2 to 7.1, whereas the isolates RS 30 from Dholi and RS 31 from Dhaula Kuan were recorded as least virulent, showed resistant disease reaction with average disease score ranged from 1.5 to 2.4. The isolate RS 29 founded to cause maximum disease intensity up to 48.2 per cent, 52.1 per cent and 49.1 per cent, followed by isolate RS 1 with 46.4 per cent, 51.4 per cent and 48.9 per cent disease intensity in maize hybrids HM 8, QPM 9 and HSC I, respectively. Whereas the minimum disease intensity was recorded in isolates RS 30 (8.7, 11.4 and 8.1% per cent) and RS 31 (8.9, 11.7 and 8.8% per cent) in maize hybrids HM 8, QPM 9 and HSC I, respectively. A wide range of host species, with variation in disease symptoms appearance, was found in Mindanao than in Luzon and fifty-two isolates belonged to anastomosis group AG1-IA showed variation in virulence

spectrum with necrotic spots and foliar blight of durian and coffee while thirty haplotypes of *R. solani* AG1-IA isolates from the Philippines and Japan clustered into seven groups of AG1-IA at the 75 per cent similarity level of RAPD fingerprint by UPGMA analysis and variation among isolates from different hosts seemed to be partially correlated with geographical origin, common origin and virulence [11]. Earlier, Mishra et al. [9] investigated the cross infectivity of the isolates collected from different hosts viz., rice, maize and green gram. All the isolates showed positive correlation in all the three hosts with variability in their pathogenicity and virulence, out of these four isolates of rice, two each of maize and green gram were found more aggressive and produced higher incidence of disease. Similarly, Singh et al.[20] revealed significant variability in the pathogenicity of *R. solani* isolates collected from different locations and classified them into highly pathogenic, moderately pathogenic and least pathogenic. A positive correlation coefficient (0.68) was found between the disease severities in relation to symptom expression. Recently, Kumar et al.[5]also observed significant variation within relative lesion height with respect to isolates Rss-12 and Rsl-1 which range from 2.0 per cent to 52.6 per cent on inbred lines (CM-600, LMDR-2, LM-12, LM-11) and hybrid (JH3459, PMH 2 and PMH 4). They also classified isolates into three groups viz., group A (highly virulent), group B (moderately virulent), group C (least virulent) based on the virulence spectrum on different cultivars.

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#### 4. CONCLUSION

Our present studies indicated that the pathogenic variability existed among the *R. solani* f. sp. *sasakii* isolates obtained from major maize growing regions of India. This demonstrated that significant pathogenic variations of the isolates within the same and different geographical locations. Comparable study of their incubation period, average disease score and disease intensity on the different maize hybrids indicates the virulence spectrum of each isolate. Virulence spectrum of this pathogen will results in improving better ways of understanding the epidemiological studies, histopathology, dissemination of pathogen and proper management of disease. Identification of isolates characterization and their pathogenic behaviour in causing infection and establishment study on host can improve the better integrated management of banded leaf and sheath blight.

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