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Virulence Spectrum among *Rhizoctonia solani* f. sp. *sasakii* Isolates from Multi-Geographical Locations of India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 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

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average disease score ranged from 2.1 to 7.1depending upon the host and isolate interaction after artificial inoculation. Disease intensity of all the RSS isolates on maize hybrids varied from 8.1 to 52.1% while isolates RSS 29 (New Delhi) and RSS 1 (Karnal) were found highly virulent, in comparison to other forty nine RSS isolates. As these isolates were highly virulent on maize hybrids with average disease score ranged from 6.2 to 7.1, whereas the isolates RS 30 (Dholi) and RS 31 (Dhaula 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 temperature regions [1]. 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 [2]. 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 [3,4,5].

The pathogen *R. solani* f. sp. *sasakii* incited the banded leaf and sheath blight disease of maize. In India, banded leaf and sheath blight disease of maize was firstly reported as a minor disease [6]. Later, it gained epidemic dimensions across Himalayas region and foot hills of Mandi district of Himachal Pradesh [7]. 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, Cost, Ivory, Sierra Leone, England, Nepal, Korea, Southern China, Philippines, Pakistan, Malaysia, Indonesia and Vietnam [8,9]. 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, Sikkam, Madhya Pradesh, Delhi, Rajasthan, Orissa, Andhra Pradesh and West Bengal [10,11,12]. 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 [13,14,15,16,17]. In view of the aforesaid, the present investigation was under taken to study the pathogenic behaviour of RSS isolates obtained from different climatic conditions of India. The finding of the study can be further used to develop suitable management strategies against BLSB.

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 min 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 $\pm 2^{o}$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 potato dextrose agar plate. The RSS isolates collected from different locations were designated as RSS 1 to RSS 51. The pure cultures were preserved on PDA slants in the refrigerator at 4°C for the further experiments.

2.3 Identification and Maintenance of the *R. solani* f. sp. *sasakii* Isolates

The isolates of 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 RSS isolates collected from major maize growing districts of India were identified and grown on potato dextrose agar (PDA) slants. These slants were sub cultures on new slants at regular time intervals and stored at 4^{o}C.

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 Unveristy, Regional Research Station Karnal. For each isolate, three hybrids (HM 8, QPM 9 and HSC I) were sown individually in separates pots with the four replications.

2.4.2 Preparation of mass culture and artificially inoculation

The mass culture of each isolate of RSS 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 RSS 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 (RSS 1 to RSS 51) was determined by inoculating on 30-35 days old plants of each hybrid. 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.

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) [18].

2.4.3.3 Disease intensity

The disease intensity was calculated using formula [19] for each isolate on each hybrid 15 days after inoculation following 1-9 rating scale [18].

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

3. RESULTS AND DISCUSSION

3.1 Characterization of *Rhizoctonia solani* f. sp. *sasakii* Isolates

A total of fifty one *R. solani* f. sp. *sasakii* (RSS) 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 51 RSS 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 Fig. 1. The incubation period of all the RSS isolates ranged from 3 to 7 days depending upon the host and isolate interaction after artificial inoculation. The shortest incubation period of eleven RSS isolates (RSS 1, RSS 2, RSS 3, RSS 7, RSS 8, RSS 10, RSS 16, RSS 23, RSS 29, RSS 37 and RSS 44) were observed in hybrid HM 8 up to 4 days, whereas the longest incubation period of 7 days was observed in four isolates (RSS 17, RSS 19, RSS 25 and RSS 33). In case of hybrid QPM 9, the shortest of incubation period 3 days were recorded in seven isolates (RSS 2, RSS 8, RSS 10, RSS 16, RSS 23, RSS 29 and RSS 44), while the longest incubation period of 7 days was found in four isolates (RSS 13, RSS 17, RSS 25 and RSS 34). Similarly, in the sweet corn hybrid HSC I, the shortest incubation period 4 days observed in eleven isolates *viz.,* RSS 1, RSS 2, RSS 3, RSS 7, RSS 8, RSS 10, RSS 16, RSS 23, RSS 29, RSS 37 and RSS 44, whereas longest incubation period of 7 days recorded in four isolates (RSS 17, RSS 19, RSS 25 and RSS 33).

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

The disease intensity of all the RSS isolates on maize hybrids varied from 8.1 to 52.1 per cent (Table 3). The isolates RSS 29 from New Delhi and RSS 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 RSS 30 from Dholi and RSS 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 RSS 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 RSS 1with 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 RSS 30 (8.7, 11.4 and 8.1%) and RSS 31 (8.9, 11.7 and 8.8%) 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 and virulence [20]. Earlier, Mishra et al. [21] 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. [22] 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. [23] 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 inbreed 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.

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

Sr. No.	Designation	Place of isolates	State
1	RSS 1	Uchani (Karnal)	Haryana
2	RSS 2	Kuchpura (Karnal)	Haryana
3	RSS 3	Indri (Karnal)	Haryana
4	RSS 4	Nissing (Karnal)	Haryana
5	RSS 5	Saha (Ambala)	Haryana
6	RSS 6	Saphera (Ambala	Haryana
7	RSS 7	Jaloli (Ambala)	Haryana
8	RSS 8	Behlon (Panchkula)	Haryana
9	RSS 9	Tikkar Taal, Morni (Panchkula)	Haryana
10	RSS 10	Bariya, Kalka (Panchkula)	Haryana
11	RSS 11	Kidarpur, Kalka (Panchkula)	Haryana
12	RSS 12	Rapouli, Mustafabad (Yamunanagar)	Haryana
13	RSS 13	Basant Pura, Radaur (Yamunanagar)	Haryana
14	RSS 14	Ladwa (Kurukshetra)	Haryana
15	RSS 15	Shahbad, Markanda (Kurukshetra)	Haryana
16	RSS 16	Behlolpur (Panipat)	Haryana
17	RSS 17	Aterna (Sonipat)	Haryana
18	RSS 18	Manouli (Sonipat)	Haryana
19	RSS 19	Bhirdana (Fatehabad)	Haryana
20	RSS 20	Darbi (Sirsa)	Haryana
21	RSS 21	Hansi (Hisar)	Haryana
22	RSS 22	Dera Bassi	Punjab
23	RSS 23	Ludhiana	Punjab
24	RSS 24	Hoshiarpur	Punjab
25	RSS 25	Udiapur	Rajasthan
26	RSS 26	Pantnagar	Uttarakhand
27	RSS 27	Almora	Uttarakhand
28	RSS 28	New Delhi	New Delhi
29	RSS 29	New Delhi	New Delhi
30	RSS 30	Dholi	Bihar
31	RSS 31	Dhaula Kuan	Himachal Pradesh
32	RSS 32	Varanasi	Uttar Pradesh
33	RSS 33	Maunath Bhanjan	Uttar Pradesh
34	RSS 34	Mirzapur	Uttar Pradesh
35	RSS 35	Barapani	Meghalaya
36	RSS 36	Kalyani	West Bengal
37	RSS 37	Ranchi	Jharkhand
38	RSS 38	Hyderabad	Telangana
39	RSS 39	Dharwad	Karnataka
40	RSS 40	Coimbatore	Tamil Nadu
41	RSS 41	Karnal	Haryana
42	RSS 42	Kaul	Haryana
43	RSS 43	Kaul	Haryana
44	RSS 44	Fatehabad	Haryana
45	RSS 45	Hisar	Haryana
46	RSS 46	Sirsa	Haryana
47	RSS 47	Karnal	Haryana
48	RSS 48	Rewari	Haryana
49	RSS 49	Hisar	Haryana
50	RSS 50	Hisar	Haryana
51	RSS 51	Hisar	Haryana

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
8.0-9.0	Susceptible (S)	88.88-99.99

Table 3. Pathogenic variability among different isolates of *R. solani* f. sp. *sasakii* (RSS)

	HM 8			QPM 9			HSC I		
Isolates	**Incubation Period (days)**	**Average Rating scale**	**Disease Intensity (%)**	**Incubation Period (days)**	**Average Rating scale**	**Disease Intensity (%)**	**Incubation Period (days)**	**Average Rating scale**	**Disease Intensity (%)**
RSS 1	4	6.6	46.4	4	7	51.4	4	6.2	48.9
RSS 2	4	6.3	42.5	3	6.7	47.5	4	5.9	44
RSS 3	4	5.4	34.3	4	5.6	37.3	4	5	33.8
RSS 4	6	3.7	15.4	6	3.9	18.4	6	3.3	14.9
RSS 5	5	4.2	39.5	4	4.4	42.5	5	3.8	38.5
RSS 6	5	4.6	35.7	6	4.8	40.7	5	4.2	36.7
RSS 7	4	2.2	11.5	4	2.6	16.5	4	1.8	12.5
RSS 8	4	5.7	42.2	3	6.1	47.2	4	5.7	43.2
RSS 9	6	3.6	19.7	6	4	24.7	6	3.6	21.2
RSS 10	4	6	44.7	3	6.4	49.7	4	6	46.2
RSS 11	5	4.7	32.5	4	5.1	38.5	5	4.7	36.5
RSS 12	5	6.2	33.1	5	6.6	39.1	5	5.8	37.1
RSS 13	6	2.6	13.2	7	3	19.2	6	2.2	17.2
RSS 14	5	4.8	36.8	4	5.2	42.8	5	4.4	40.8
RSS 15	5	5.3	35.4	5	5.7	41.4	5	4.9	39.4
RSS 16	4	5.6	43.2	3	6	49.2	4	5.7	47.2
RSS 17	7	3.1	16.5	7	3.5	22.5	7	3.2	20.5
RSS 18	6	2.8	10.2	6	3.2	15.2	6	2.9	10.5
RSS 19	7	2.3	9.6	6	2.7	14.6	7	2.4	9.9
RSS 20	6	3.5	25.3	6	3.9	30.3	6	3.7	25.6
RSS 21	6	3.7	27.4	4	4.1	32.4	6	3.9	27.7
RSS 22	5	4.9	33.3	5	5.2	38.3	5	5	34.8

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 (%)
RSS 23	4	6.4	47.3	3	6.7	49.3	4	6	45.8
RSS 24	6	3.1	21.6	6	3.4	23.6	6	2.7	20.1
RSS 25	7	2.3	12.7	7	2.6	14.7	7	1.8	11.2
RSS 26	6	3.5	19.5	6	3.8	21.5	6	3	17.2
RSS 27	6	3.7	20.1	6	4	22.1	6	3.2	17.8
RSS 28	6	2.9	18.3	6	3.2	20.3	6	2.3	16
RSS 29	4	6.8	48.2	3	7.1	52.1	4	6.2	49.1
RSS 30	6	2.1	8.7	6	2.4	11.4	6	1.5	8.1
RSS 31	6	2.1	8.9	6	2.4	11.7	6	1.6	8.8
RSS 32	6	2.6	20.1	6	2.9	25.1	6	2.1	21.6
RSS 33	7	3	28.5	6	3.3	33.5	7	2.5	30
RSS 34	6	3.6	22.6	7	4	29.6	6	3.2	26.1
RSS 35	6	3.1	25.5	6	3.5	32.5	6	2.7	29
RSS 36	6	3.7	28.2	6	4.1	35.2	6	3.7	30
RSS 37	4	6.1	43.8	4	6.5	52.8	4	6.1	42.6
RSS 38	6	2.8	26.5	6	3.2	33.5	6	2.8	28.3
RSS 39	5	5.1	36.9	4	5.5	41.9	5	4.7	36.7
RSS 40	6	2.4	10.6	6	2.8	15.6	6	2	10.4
RSS 41	5	5.3	39.1	4	5.7	44.1	5	4.9	38.9
RSS 42	5	5.7	37.5	4	6.2	42.5	5	5.4	39
RSS 43	6	2.7	26.4	6	3.2	31.4	6	2.4	27.9
RSS 44	4	6.1	44	3	6.4	47.7	4	5.6	44.6
RSS 45	6	3.4	21.1	6	3.7	22.1	6	3.1	19
RSS 46	6	3.3	19.4	6	3.6	20.4	6	3	17.3
RSS 47	5	3.4	14.3	4	3.9	15.3	5	3.3	12.2
RSS 48	5	4.7	19.3	4	5.1	24.3	5	4.5	21.2
RSS 49	5	3.2	13.4	5	3.6	17.4	5	2.8	13.9
RSS 50	6	3	13.1	6	3.4	17.1	6	2.6	13.6
RSS 51	5	5.3	37.5	5	5.7	41.5	5	4.9	38
RSS 49	5	3.2	13.4	5	3.6	17.4	5	2.8	13.9
RSS 50	6	3	13.1	6	3.4	17.1	6	2.6	13.6
RSS 51	5	5.3	37.5	5	5.7	41.5	5	4.9	38

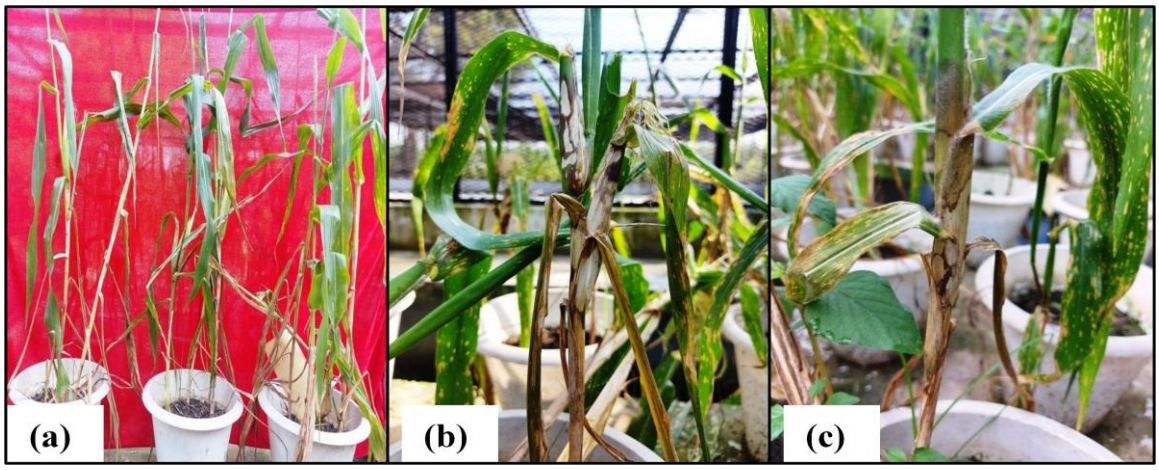

Fig. 1. Pathogenic variation in typical symptoms (a-c) among different isolates of *R. solani* on different maize hybrids under screen house conditions

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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