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First report of *Rhizoctonia solani* causing Banded leaf and sheath blight disease in Sugarcane (*Saccharum officinarum*) from Ghazipur district of Uttar Pradesh, India

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Globally, sugarcane (*Saccharum officinarum* L.) is probably the most ancient and one of the important crops cultivated in the tropical and subtropical countries for the production of sugar, ethanol and other products including molasses, falernum, cachaça, rum and bagasse. In some parts of the world people use sugarcane reeds to make mats, pens, thatch and screens (Anonymous 2017, 2018). India is the second largest sugarcane producing country (4.6 mha with a production of 2.95 MT) after Brazil (5.34 mha with a production of 3.86 MT) accounting for 40% global sugarcane production (FAO 2012). Approximately, 79% of the sugar produced worldwide comes from *Saccharum officinarum* and other hybrids of this species, and the rest is made from sugarbeet. Sugarcane predominantly grows in tropical and subtropical regions; However, sugar beet typically grows better in regions with comparatively cooler climates. In India, Uttar Pradesh has the largest area under cane production (~50% of the cane area in the country) followed by Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Gujarat, Bihar, Haryana and Punjab (Anonymous 2017, 2018). Sugarcane is affected by a number of biotic and abiotic stresses causing significant yield losses every year. Among the biotic stresses, numerous pathogens such as *Colletotrichum falcatum* inciting red rot disease*, Fusarium moniliforme* causing *pokkahboeng,* bacterial pathogen *Xanthomonas axonopodis* causing gumming disease, *Phytoplasma* causing sugarcane grassy shoot disease, and *Sporisorium scitamineum* causing whiptail disease or sugarcane smut affect cane production. Further, several viral diseases affecting sugarcane include sugarcane mosaic virus, sugarcane yellow leaf virus and maize streak virus (Gonçalves *et al.* 2011).

During the course of inter-culture operation and tying of the sugarcane (*cv*. BO-91), a typical symptom was observed for the first time in the month of August 2016 which was recorded to progress subsequently with conspicuous characteristics having the presence of peculiar bands on sugarcane leaf and leaf sheath. Symptoms were observed on all the aerial plant parts except tassel. However, no visible symptoms were observed in the roots. The disease manifests itself on leaf sheaths, leaves and canes as banded leaf and sheath blight (Fig 1A). In the severely infected plants, stalk lesions or rind spotting with banding of top leaf resulting in head rots were observed. Sclerotial bodies on affected parts of the plants were also observed (Fig 1A).

Looking into the disease progression and severity in the sugarcane field of Shri Mahatam Singh (Village Bhawanipur, Post Kansahari, District Ghazipur, Uttar Pradesh, India), an exploration survey was planned to study the disease spread in other parts of Ghazipur district with Mr. Bhanu P. Singh in the month of September, October and November 2016 and 2017. The infected plant samples were collected from different parts of the district. A total of 15 samples were collected and brought to the laboratory for the isolation of causal organism(s) (Table 1). For the isolation of causal organisms, a small infected portion of leaf, leaf sheath and stalk were collected, surface sterilized using 2% sodium hypochlorite solution (1 min) and subsequently rinsed with sterile distilled water thrice. The surface sterilized plant parts were placed on Petri dishes containing potato dextrose agar and nutrient agar medium and incubated for 5–7 days at 27±2□. However, collected sclerotia were surface sterilized using 1% sodium hypochlorite solution (1 min)

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Fig 1A Infected sugarcane plants showing symptoms of caused by *Rhizoctonia solani*, (i) showing infected leaves and sheath and (ii, iii) showing infected top leaf with developing fungal sclerotia on the surface.

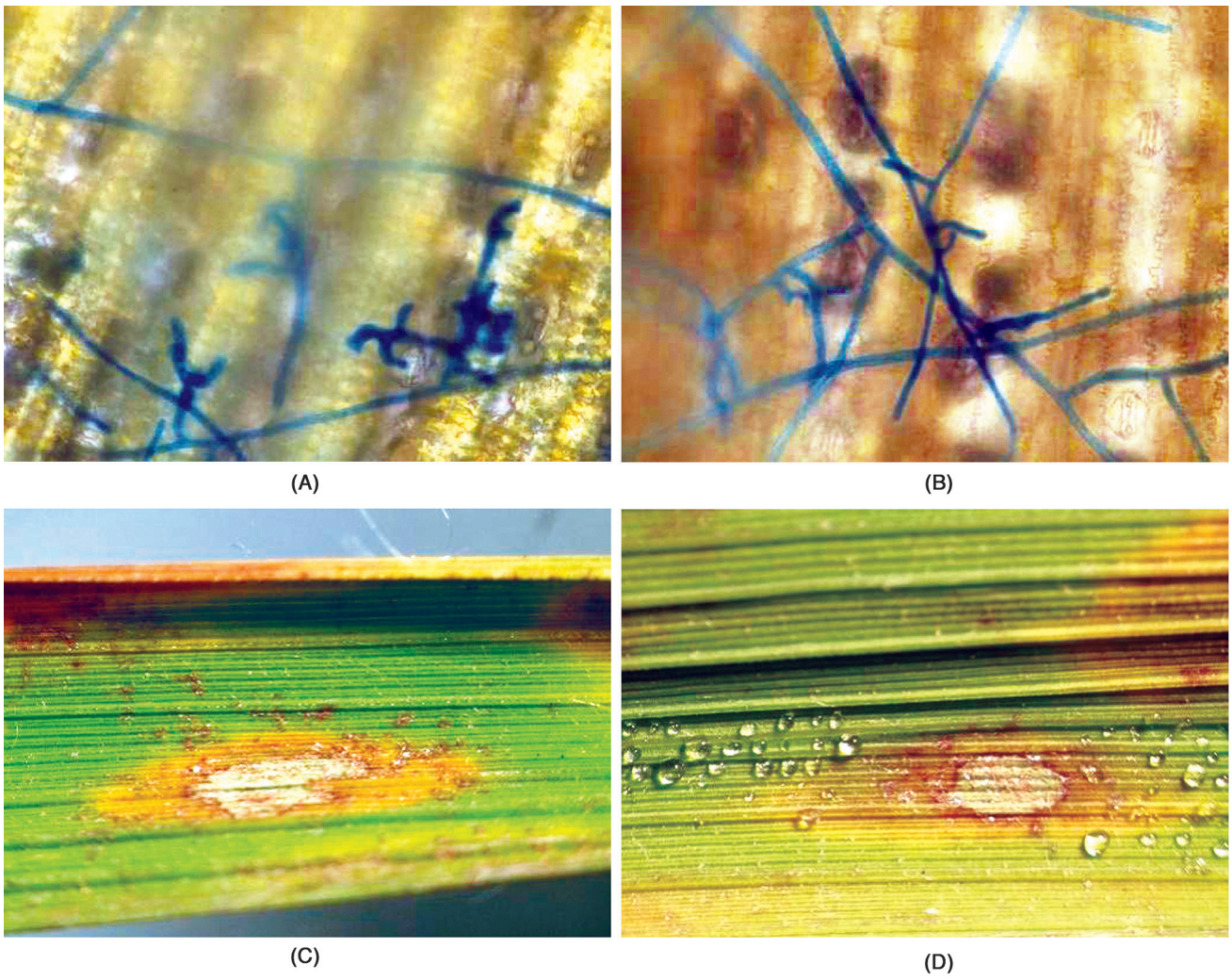

Fig 1B Colonization of *Rhizoctonia solani* isolates on sugarcane leaf (A) SCRS-2018-1A and (B) SCRS-2018-1B and development of water-soaked lesions on sugarcane leaf by (C) SCRS-2018-1A and (D) SCRS-2018-1B during pathogenicity test under *in vitro* conditions.

and subsequently rinsed thrice with sterile distilled water. These surface sterilized sclerotia were cut into small pieces using sterile scalpel and placed on Petri dishes containing potato dextrose agar medium and incubated for 7 days at 25±1□. After 3rd day of incubation, white translucent mycelial growth was observed in all the samples on potato dextrose agar medium. After 7 day of incubation, the fungus was purified using hyphal tip culture method. The similar type of fungal growth was also observed in the Petri dishes inoculated with fungal sclerotia on 5th day of incubation. However, in 5 samples bacterial colonies were observed on 4th day of incubation and they were purified using quadrat streaking on nutrient agar at 27±1□. A total of 15 fungal and 5 bacterial isolates were isolated from sugarcane grown at different locations and they were morphologically similar on potato dextrose agar medium.

Pathogenicity test of the fungal and bacterial isolates was conducted following Koch's postulates. For this, fungal isolates were grown on barley (*Hordeum vulgare* L.) grains. Barley seeds (50 g) were moistened with 60 ml tap water in a 250 ml conical flask, plugged with non-absorbent cotton and autoclaved for 20 min at 121□ (15 pound per square inch). Flasks were inoculated with each fungal isolate separately and incubated for 20 days at 25±1□. During course of incubation, barley grains were covered with fungal mycelia and numerous sclerotia developed in the flasks. However, bacterial strains were inoculated in the flask containing nutrient broth and incubated for 5 days at 27±1□. To check the pathogenicity, grain-based formulation containing fungal mycelia and sclerotia was inoculated in sugarcane plants (*cv*. BO-91) in between the sheath and stalk of the 2nd, 3rd and 4th internode and tied with cotton threads. However, bacterial suspension (1 ml) was inoculated between the sheath and stalk as mentioned above in August, 2018 at the experimental field of the corresponding author. The relative humidity was 85–90%. After 15 days of inoculation, symptoms appeared as irregular, water-soaked, straw-coloured lesions on sheath and leaf bases. In due course of time, lesions enlarged rapidly and converted into dark bands as appeared in naturally infected plants. However, no symptoms were observed in the bacteria inoculated plants. Further to re-confirm, sugarcane setts (30 cm) (with leaf sheath attached) were brought to laboratory, surface sterilized with 70% ethyl alcohol and inoculated with fungal and bacterial isolates. After inoculation, these setts were kept in a beaker containing nutrient (Hoagland) solution. To maintain the humidity, sterile water was sprayed at 4 h interval with the help of hand sprayer. After 7 days of inoculation, irregular water-soaked lesions were observed. The peculiar symptom of banded leaf and sheath blight was observed at 15 days of fungal inoculation (Fig 1B). However, no visible symptom was observed in the bacteria inoculated setts. Fungus from artificially induced diseased plants showing a typical symptom was re-isolated to confirm the pathogenicity of the causal organism *in vitro*. Such re-isolated fungal isolates were morphologically similar to those isolated from natural infection.

Identification of the fungal pathogen was done after it was grown on potato dextrose agar medium. The colonies produced by the fungus were comparatively fast growing and formed silky white colonies on potato dextrose agar medium at 25±1□, losing their luster gradually and becoming dull in appearance (Supplementary Fig 1 A-B). Further, the mycelium was characterized as pale to brown on the potato dextrose agar medium. The branching was observed near the distal septum in young growing hyphae at right angles (Supplementary Fig 1 C-D). The young multinucleate hyphal cells with a predominant septal pore apparatus, presence of a constriction and formation of a septum in branch near the point of origin were the other important characteristics observed. These isolates were found to produce sclerotia which are of undifferentiated texture. Based on the cultural

Table 1 Samples and sampling site explored for isolation of pathogen *Rhizoctonia solani* from infected sugarcane plants

Sample No.	Sample type	Sampling site	Sampling year
Sample-01	Infected leaf and sheath	Bhawanipur, Kansahari, Ghazipur	2016
Sample-02	Infected ear head with sclerotia	Bhawanipur, Kansahari, Ghazipur	2016
Sample-03	Infected leaf and sheath	Nandganj, Ghazipur	2016
Sample-04	Infected leaf and sheath	Nonahara, Ghazipur	2016
Sample-05	Infected leaf and sheath	Saidpur, Ghazipur	2016
Sample-06	Infected leaf and sheath	Ghariha, Ghazipur	2016
Sample-07	Infected leaf and sheath	Salamatpur, Ghazipur	2016
Sample-08	Infected ear head with sclerotia	Jamaniya, Ghazipur	2016
Sample-09	Infected ear head with sclerotia	Karanda, Ghazipur	2017
Sample-10	Infected leaf and sheath	Sadat, Ghazipur	2017
Sample-11	Infected leaf and sheath	Haridaspur, Ghazipur	2017
Sample-12	Infected leaf and sheath	Bhawanipur, Kansahari, Ghazipur	2017
Sample-13	Infected ear head with sclerotia	Nandganj, Ghazipur	2017
Sample-14	Infected leaf and sheath	Saidpur, Ghazipur	2017
Sample-15	Infected ear head with sclerotia	Sadat, Ghazipur	2017

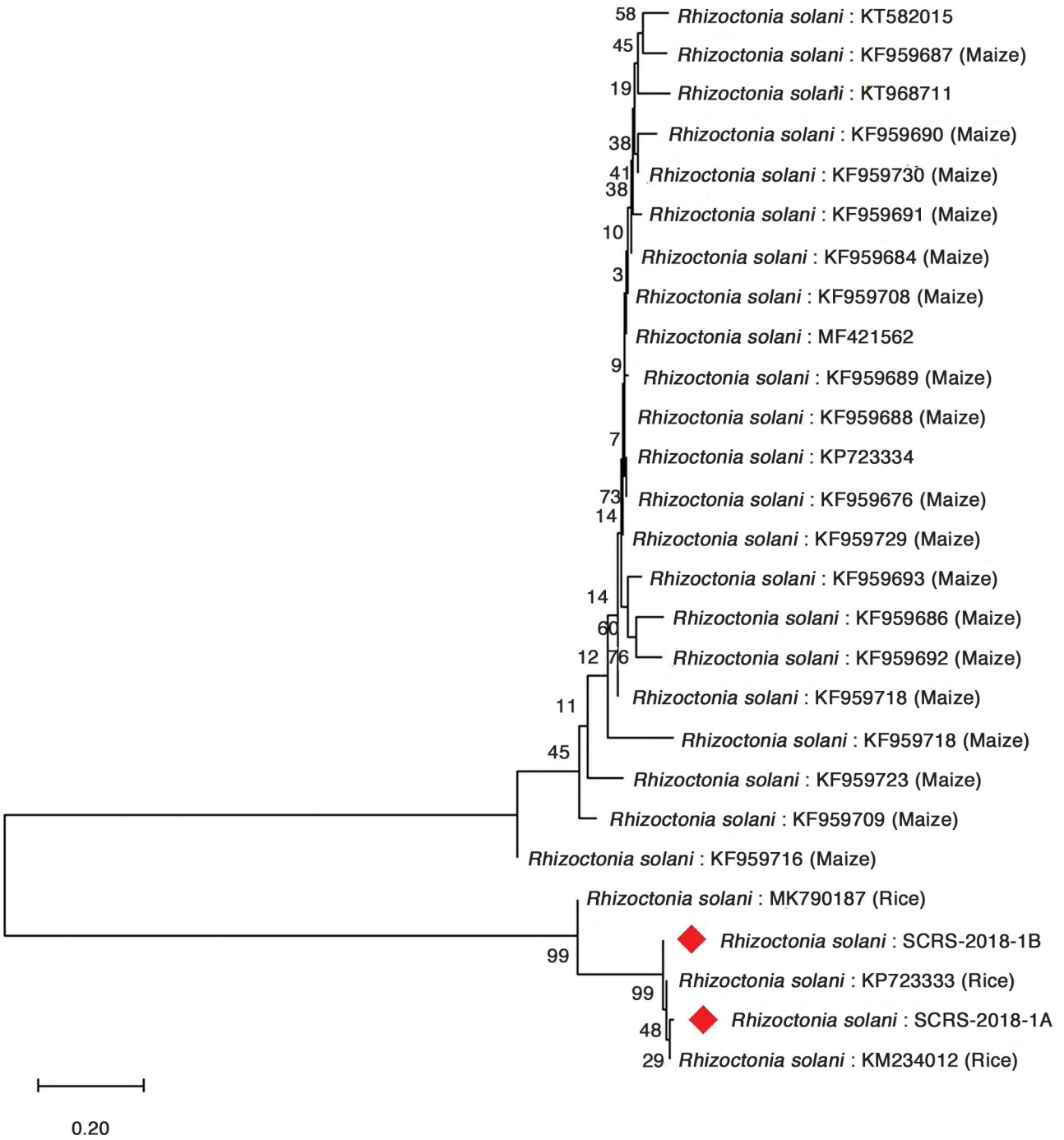

Fig 2 Neighbour joining tree derived by CLUSTAL W and MEGA X using analysis of ITS sequences showing the nearest match. The numbers at nodes indicate bootstrap support values, as calculated by MEGA 5.0.

and morphological characters the fungus was tentatively identified as *Rhizoctonia solani*.

For molecular level identification, the two most virulent strains (SCRS-2018-1A and SCRS-2018-1B) were grown on potato dextrose broth for 7 days and mycelial mats were harvested separately. The fungal DNA was isolated using Nucleo-Pore Genomic DNA Isolation Kit (Genetix Pvt. Ltd.) following the manufacturers protocols. DNA sequences of the internal transcribed spacer region (ITS) were amplified using ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) universal primers (Bokulich and Mills 2013) and sequenced. The representative ITS sequences of strains SCRS-2018-1A and SCRS-2018-1B were searched on BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST search results indicated that ITS sequence of strain SCRS-2018-1A showed 99.85% similarity with ITS sequence of *Rhizoctonia solani* (Accession No. MG397063) deposited at NCBI GenBank. However, ITS sequence of strain SCRS-2018-1B showed 100% similarity with *R. solani* Accession No. MN535026.

The ITS gene sequences were submitted to NCBI GenBank and the Accession numbers MT239050 and MT239051 were obtained. Further, highly similar sequences were downloaded, dendrogram was prepared and nearest match was analysed. Both the fungal isolates were identified as *R. solani* (Fig 2). To our knowledge, this is the first report of *R. solani* causing banded leaf and sheath blight disease in Sugarcane from Ghazipur district of Uttar Pradesh (India) which needs an immediate attention.

SUMMARY

During August 2016, banded leaf and sheath blight symptoms were observed on sugarcane (*cv.* BO-91) in the Village Bhawanipur of Ghazipur district of Uttar Pradesh (India). A survey was done to assess the prevalence of and collect the specimens of banded leaf and sheath blight disease from sugarcane fields in the Ghazipur district during 2016–17. A total of 15 fungal isolates were collected from different parts of the district and pathogenicity testing of these isolates was done following Koch postulates. All the 15 isolates were able to produce disease in the controlled laboratory conditions as well as in the field. Further, these isolates were morphologically characterized as *Rhizoctonia solani*. To confirm the identity at molecular level, ITS sequencing of two most virulent isolates was done. Based on the ITS sequence similarity, these two isolates, viz. SCRS-2018-1A and SCRS-2018-1B were identified as *R. solani.* As per the existing records and our knowledge based on the extensive survey of pertinent literature, this is the first report of *R. solani* causing banded leaf and sheath blight disease in Sugarcane from Ghazipur district of Uttar Pradesh, India.

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