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Characterization of pathogen associated with the banded leaf and sheath blight disease of maize in Gujarat, India

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Banded leaf and sheath blight a soil-borne disease caused by a *Rhizoctonia solani* f. sp. *sasakii*(teleomorph: *Thanatephorus cucumeris*) is one of the very destructive diseaseswhich under favourable environmental conditions cause substantial yield losses. Based on cultural, morphologicalcharacteristics and sequence analysis of the Internal Transcribed Spacer (ITS), the pathogen associated with the banded leaf and sheath blight of maize was identified as *Rhizoctonia solani* f. sp. *sasakii*. Thedisease sample was collected from Main Maize Research Station, Godhra (22.77^0 N latitude, 73.62^0 E longitude), Gujarat, India.

Keywords : Banded leaf and sheath blight – *Rhizoctonia solani* f. sp. *sasakii*

INTRODUCTION

Maize (*Zea mays* L.) is the most important cereal crop belonging to the grassy family Poaceae (Gramineae) tribe Maydeae and is the sole domesticated and economically important member of the genus Zea. The center of origin of the crop is supposed to be Central America. Being one of the most important crops in the world agricultural economy, it occupies the third position in the world after wheat and rice in area and production. However, as far as productivity is considered it ranks first. Maize has approximately 72 per cent starch, 10 per cent protein and 4 per cent fat (Nuss and Tanumihardjo, 2010). In maize, 112 different diseases were reported from different parts of the world. Among these, 65 diseases are reported from India. The major diseases in different agro-climatic regions are seedling blight, seed rots, stalk rots, leaf spot and blight, downy mildew, banded leaf and sheath blight, smuts and rusts which causes around 15-20 per cent yield loss each year (Saxena, 2002). Among them, banded leaf and sheath blight (BLSB) of maize caused by *Rhizoctonia solani* f. sp. *sasakii* Exner. This disease was firstly observed in Ceylon and they gave the name sclerotial disease of maize (Bertus, 1927).

This disease was first time observed in the Tarai region (foothill plain area) in Uttar Pradesh. This pathogen is soil-borne and its occurrence has also been recorded in several maize growing areas from Himachal Pradesh, Uttar Pradesh, Haryana, Punjab, Madhya Pradesh, Rajasthan and Gujarat.

In India, losses in grain yield have been estimated in the range of 11 to 40 per cent, even to 100 per cent (Madhavi *et al*. 2011; Gao *et al*. 2014; Izhar and Chakraborty, 2013; Hooda *et al*.2017). It is a very destructive, most prevalent and versatile pathogen that infects rice, soybean and alfalfa like many plants, including maize. *R. solani* is a genetically diverse group and it has more than 100 species that attack several crop plants (Binder*et al*.,2005). The sexual stage of *R. solani* is *Thanatephorus sasakii*. The pathogen does not produce spores on maize and it is identified by characteristic mycelium and sclerotia. This fungus produces dolipore septate mycelium. It produces a large number of spherical brown

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sclerotia. Clamp connection and conidia are absent, but monilioid cells are present. Right angle branched mycelium and constriction are observed.

Due to the banded leaf and sheath blight disease and based on the above facts, the present investigation was carried out to identify the *Rhizoctonia* species associated with the disease in Gujarat (India). Pathogens were characterized microscopically and molecularly, to determine the extent of the disease and damage where the disease prevails.

MATERIALS AND METHODS

Collection of Diseased Samples

The leaves showing typical symptoms of banded leaf and sheath blight, caused by*R. solani* f. sp. *sasakii* were collected from maize fields of Main Maize Research Station, AAU, Godhra. The freshly collected diseased leaves, sheaths and stalks of maize showing banded leaf and sheath blight with characteristic water-soaked, discoloured concentric bands and rings, often brown, tan or grey in coloured symptoms (Fig. 1) brought from field during *Kharif,* 2021 were thoroughly washed under running tap water and then immediately examined under a compound microscope for presence of the pathogen. Later on, the fungus was isolated and maintained as pure culture on potato dextrose agar using following method.

Isolation of the pathogen

Fresh infected leaves/sheath tissues of maize bearing fungal sclerotia and showing characteristic BLSB symptoms were surface-sterilized with 1% sodium hypochlorite for 1 min. and incubated on Potato Dextrose Agar (PDA) by using standard tissue isolation procedure was given by (Tuite, 1969). The pure cultures of the pathogens from the pathogen mixture were obtained by the hyphal tip method (Rangaswami, 1972).

Cultural and morphological characterization

Identification of pathogen causing BLSB of maize grown on PDA medium was examined visually as well as microscopically for cultural and morphological characters. The cultural characteristics were recorded right from the initiation of growth up to 15 days including colony growth, colony colour, colony texture as well as sclerotia arrangement, sclerotia weight was measured and size of sclerotia was measured using ocular micrometre. The morphological characters *viz*., length and width of mycelium, mycelium septation and mycelium arrangement were measured under a microscope with high power magnification from 10 days old culture of *R. solani* f. sp. *sasakii*. Identification was done after comparing the microscopic and morphological features of the pathogenic fungi with the available standard literatures (Singh and Shahi, 2012; Akhtar *et al*. 2009).

Molecular characterization

The total genomic DNA was isolated from fungal sclerotia using cetyl trimethylammonium bromide (CTAB) DNA extraction protocol. PCR was performed with the respective primers (Table 1) for amplification of the internal transcribed spacer region of ribosomal DNA (rDNA-ITS region) for pathogens the sequencing of the amplified products was carried out in an automated sequencer ABI 3730 genetic analyzer at Eurofins Genomics India Pvt. Ltd., Bengaluru, Karnataka. Sequences were used in a BLASTN search of the nucleotide database at NCBI (https://blast.ncbi.nlm.nih.gov) to confirm the species associated with the disease. Genetic variations among the isolates were also analysed using BioEdit version 5.09 and a phylogenetic tree was constructed using MEGA X software.

Pathogenicity Test

The pathogenicity of the pathogen was proved by artificial inoculation of pathogen by following standard method of inoculation i.e., Koch's postulates as follows: In order to study the pathogenicity of the test fungus on maize plants, two-week old seedlings of susceptible maize cultivar CM600 were used. The test seedlings were grown in earthen pots. Autoclaved soil was filled in earthen pots and inoculated with pathogen, *R. solani* f. sp. *sasakii*. The fungus multiplied on sorghum grain in flasks was added

@ 100g/2kg of soil. Five seeds were sown in each pot. The initial seed germination count was recorded.

Regular monitoring was done for observation of symptom development. The disease development was noted up to 60 days of sowing or until symptom development. The pathogen was re-isolated from the infected plants and cultured on PDA. The obtained culture was compared with that of original culture obtained from the infected plants from the field.

RESULTS AND DISCUSSION

Isolation and Purification of Pathogen

The samples of diseased leaves from infected maize plants were collected from Main Maize Research Station, AAU, Godhra. The isolation was performed as described in materials and methods. The symptoms *viz*. stalk lesions (rind spotting), stalk breakage, clumping and caking of styles (silk fibers), horseshoe-shaped lesions with banding on caryopses and sclerotial formation on styles, glumes, cupules and caryopses are typical considered for visual identification of banded leaf and sheath blight disease were used as identification for isolation of the fungus. The pathogenic fungus was isolated on the PDA medium and purified by single sclerotia isolation method. The pure culture was maintained on PDA medium for further study.

Cultural characteristics of Rhizoctonia solani

On PDA medium, fungus grew fast and formed silky white colonies at 28±2 °C. After five to six days, the colony gradually lose their lustre and became dull in appearance. The mycelium was colourless when young, but assumed brown colour as it matured(Fig.2).

Morphological characteristics of Rhizoctonia solani

The microscopic observation revealed that the mycelium was septate and branching at right angles. Hyphae was found to be septate and typically constricted at the point of branching(**Fig.2**). The inter septate region measured 25.18-48.24 x 8.5-12.98 µm on average.

Rhizoctonia solani f. sp. *sasakii* was generally identified by characteristics of the mycelium and sclerotia. The mycelium was colourless when young, but becomes brown colour as it matures, on PDA medium, *R. solani* f. sp. *sasakii* produced white to deep brown, cottony mycelium. Under microscopic examination, hypha was multinucleate, septate and branching at right angles and acute angles approaching 45Ú. Hyphae were typically constricted at the point of branching and contain dough nut shaped pore that enables nuclei and mitochondria to migrate between cells. Vegetative diameter of the hyphae was 3 to 17 µm. Sclerotia were produced abundantly in culture and on infected plant parts. Mostly, sclerotia were 1 to 5 mm in diameter with spherical shape and dark brown to black in colour(Singh and Shahi, 2012; Akhtar*et al*. 2009).

Pathogenicity Test

The sclerotia body of isolated fungus was inoculated on susceptible maize cultivar “CM600” at a 3-4 leaf stage grown in pots. The

Cultural characteristics	
Colony growth	90 mm diameter on 7th day on PDA at 28±2 °C
Colony colour	White
Colony texture	Cottony fluffy growth
Sclerotia arrangement	Sclerotia either scattered or formed a ring in centre or periphery of the plate

Morphological characteristics	
Mycelium	Branched at 90°, septate and constricted at the point of branching
Sclerotia	Irregular shaped, brown in colour, number of sclerotia 46 -113 per plate, size ranging from 0.5-4.3 mm and weight ranging from 0.3-6.1 mg.

Table 1: Details of primer sequences used for amplification of rDNA-ITS region

Primers	Sequence	Reference
Forward primer ITS 1	5´-TCCGTAGGTGAACCTGCGG-3´	White *et al.*, 1990.
Reverse primer ITS 4	5´-TCCTCCGCTTATTGATATGC -3´	

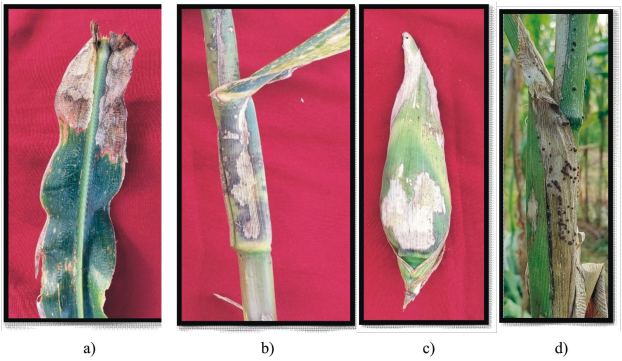


Fig 1: Symptoms on maize leaves: Leaf and sheath blight (a,b);infected cob (c); sclerotia on infected maize plant (d)

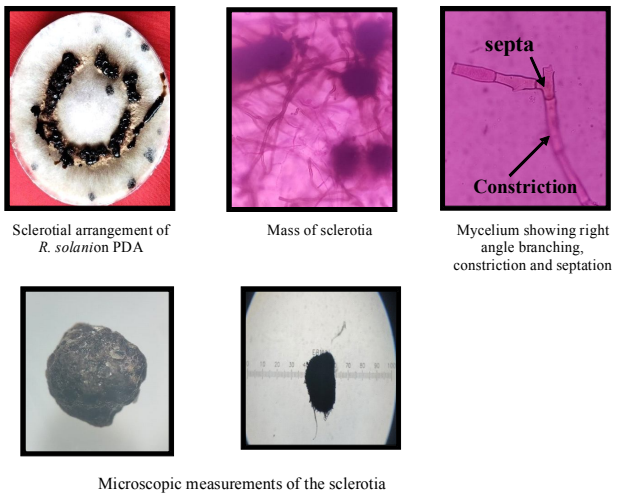
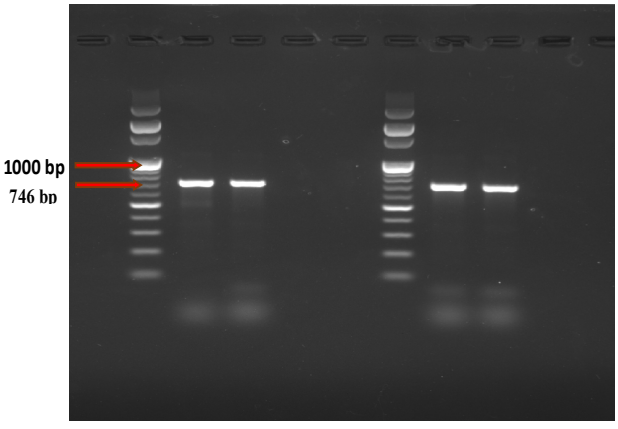


Fig. 2: Cultural and morphological characters of *R. solani*f. sp. *sasakii*.



M: 100bp DNA ladder, A: *Rhizoctonia solani*

Fig. 3: Gel electrophoresis of DNA amplicons from PCR assays using primer set ITS1 and ITS4 that amplify 746 bp region of ITS rDNA for *Rhizoctonia solani*

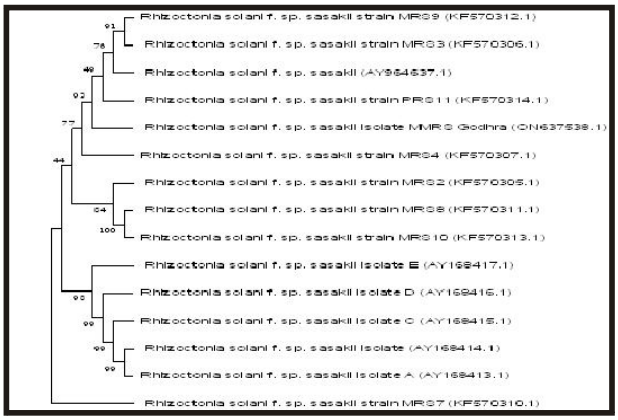


Fig. 4: Phylogenetic tree based on sequences of rDNA-ITS region of *R. solani* f. sp. *sasakii*

characteristic symptoms appeared seven to ten days after inoculation as stalk lesions (rind spotting), stalk breakage, clumping and caking of styles (silk fibres) and horseshoe-shaped lesions with banding on caryopses. After that, re-isolation was carried out from these infected leaf lesions and the culture obtained from this reisolated colony was compared with original culture of the fungus to confirm identity of the pathogen. Thus, the pathogen *R. solani* f. sp. *sasakii* was confirmed by pathogenicity test.

Molecular characterization

The amplified rDNA-ITS region of 746 bp from genomic DNA was sequenced and submitted to NCBI GenBank (ON637538.1), which showed >95% nucleotide similarity with *R. solani* f. sp. *sasakii*.

The phylogenetic trees were constructed with nucleotide sequences of the sequenced ITS regions of *R. solani* f. sp. *sasakii* and compared with other similar worldwide fungal isolates available in the NCBI database (Fig. 3).

CONCLUSION

Based on cultural and morphological characteristics and ITS rDNA sequencing, it is concluded that *R. solani*f. sp. *sasakii* (Accession

No. ON637538.1), is responsible for causing BLSB diseases of maize in Gujarat.The isolation from disease plant parts revealed the association of *R. solani* f. sp. *sasakii* which further satisfied Koch's postulates.

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DECLARATION

Conflict of interest.Authors declare no conflict of interest.

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