SHORT COMMUNICATION

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Rice stem rot *Sclerotium hydrophilum* Sace isolated and characterized in Southern Karnataka

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ABSTRACT

In the summer season of 2016 and 2017, in some fields of Cauvery command area of Karnataka the rice crop showed stunted growth with brownish to black lesions without distinct margin on stem at the water level during tillering and reproductive phase . The causal organism was tissue isolated on potato dextrose agar, colonies of pure cultures showed white mycelium at first and after two days white coloured scelerotial formation started which later changed to chocolate brown and after five days became black colour. The hyphal width was measured to be within a range of 6 to 10 «m and binucleate hyphae were observed between the septa. Small, globose and reddish brown to black coloured mature sclerotia measuring 0·24-0·43 mm were observed in culture. Koch's postulates was proved by inoculating five days old culture disc containing sclerotia on the stem of 6 week-old healthy rice plants. After seven days symptoms were observed on the inoculated seedlings but not on control plants. The fungus was re-isolated and similar cultural characteristics were observed as of the original culture. DNA fingerprinting of the rDNA-ITS sequence of the representative isolate MNDSR01 (Gene Accession MH393598.1) matched with that of Sclerotium hydrophilum reference (MH393598) with 100% identity. To our knowledge, this is the first report of S. hydrophilum in the Cauvery command area of Karnataka.

Key words: *Rice, stem rot, rDNA, sclerotia, Sclerotium hydrophilum*

Rice sheath and stem diseases caused by *Rhizoctonia* and *Sclerotium* species are problems in rice growing regions of Southeast Asia. Stem rot of rice caused by *Sclerotium hydrophilum* has been reported in rice growing countries in Southeast Asia (Kimiharu et al., 2004), Myanmar (Aye et al., 2009), North-East China (Zhong et al., 2018), Turkey (Demecri et al., 2009) and India (Singh et al., 2002; Kumar et al., 2003; Pramesh and Guruprasad, 2014; Gopika et al., 2016). Stem rot of rice incited by sclerotial fungi such as *Sclerotium oryzae* Catt., *Sclerotium hydrophilum* Sacc. and *Sclerotium oryzae* var. irregular Roger was a common disease in rice growing areas of India. (Pramesh and Guruprasad, 2014). It was more destructive in Punjab, Haryana and Tamil Nadu (Padmanabhan, 1974; Ahuja et al., 1981), particularly on high yielding varieties (Jaya, BPT 5204 and Jyothi). In Karnataka, stem rot caused by *Sclerottium oryzae* is reported in north eastern Karnataka with highest incidence of 29.55 and 14.33 per cent in Gangavathi taluk during *kharif* 2014 and 2015 respectively (Pramesh et al., 2017) in Tunga Bhadra command area. However, stem rot caused by *S. hydrophilum* Sacc. is not yet reported of Southern Karnataka.

During summer season of 2016 and 2017 in many fields of Mandya district of Karnataka, the rice crop howed stunted growth at maximum tillering and reproductive phase with distinct symptoms on stem consisting of brownish to black lesions without distinct margin that expanded and girdled the sheath were observed at the water level. The pathogen was isolated following tissue isolation. Infected leaf sheaths were surface disinfected for one minute in 0.1% mercuric chloride and transferred to potato dextrose agar (PDA)

and incubated at 28°C for seven days. White fungal colony growth started after one day of inoculation on RDA. The fungal colonies of pure culture started producing white coloured scelerotia after three days later changed to chocolate brown by two days and finally black colour after six days of inoculation (Fig. 1). The hyphal width was within a range of 6 to 10 µm and binucleate hyphae were observed between the septa. Small, globose and reddish brown to black coloured mature sclerotia measuring 0·24-0·43 mm were observed in culture. To confirm the pathogenicity, ten rice sheath tissues of 60 days old plant were inoculated by placing a five days-old mycelial plug along with sclerotia on the stem 1 cm below the axil of the fully matured leaf and wrapping with parafilm. Control plants were treated in the same manner using a plug of 2% water agar. After seven days, symptoms on the inoculated plants were similar to those naturally occurring (Fig. 2). Control plants did not develop symptoms. The fungus was re-isolated from inoculated plants, and cultural characteristics were studied which found to be very much similar to the original culture confirming Koch's postulates. The histological observations revealed brownish runner hyphae, and lobed hyphopodia were produced on the surface of infected leaf sheaths by *S. hydrophilum*. *Sclerotium hydrophilum* has been recorded previously on rice in Venezuela (Cedeno et al., 1997), in Australia (Lanoiselet et al., 2002) and in Myanmar (Aye et al., 2009).

Cultural characteristics of of *S. hydrophilum* was studied on different culture media *viz*., Potato dextrose agar, rice flour agar, V8 juice agar and oat meal agar. Among the different media maximum growth of 90 mm was recorded in V8 juice agar followed by rice flour agar and oat meal agar with 85 mm and 60 mm after five days of inoculation. PDA medium was less supportive with 45 mm growth after 4 days of inoculation.

Fig. 1. Sclerotium hydrophilum grown on Potato dextrose medium.

Fig. 2. Symptoms expressed after inoculation of *Sclerotium hydrophilum*

Molecular characterization

Molecular characterization of the isolate MNDSR01 was carried out based on the nucleotide sequencing of ITS region. DNA from the isolate was extracted following the procedure described by Lee and Taylor (1990) using universal primers ITS1 (5_-TCCGTAGGTGAACCTGCGG-3_) and ITS4 (5_-TCCTCCGCTTATTGATATGC-3_) were used to amplify the ITS regions as described by White et al. (1990) followed by PCR was performed on Eppendorf master cycler, using the following parameters: initial denaturation for 3 min at 94°C, 32 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1.5 min, and a final extension at 72°C for 8 min. The PCR products were purified after separation on a 1.5% agarose gel with the EZ spin column. Sequencing of the PCR product was performed in both directions using the BigDye Terminator Cycle Sequencing system (Applied Bio systems) USA and analysed with ABI 3100 analyzer capillary machines. Nucleotide homology searches were performed with the nucleotide program BLAST (http://ncbi.nlm.nih.gov/) confirm the identity of the pathogen.

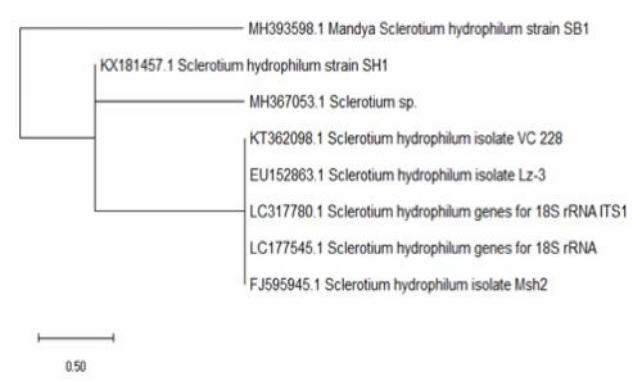

Fig. 3. Dendrogram depicting the relationship of *Sclerotium hydrophilum* and the closest related sequences of the isolates existed at NCBI

The rDNA-ITS sequence of a representative isolate MNDSR01with following FASTA format (Gene Accession MH393598.1)

>TACCCCGTCCCTGTCTGTGTCACCCATCCCCACCCTCTCTCTCTCATCACACACACACACACACAC
CTGTGAACTTGTGAGACCCGAACCCAAAGAAGAGATCCTACCTTTCCGTCTACTCAATCCATTAC
ACAAACCCCAATTGATATTTAAACTGAATGTAATTGTGATGTAACACATCATTAGAAACTAAGTTTCA
ACAACCGATCTCTTCCCTCTCCCATCGATGAAGAACCCAGCCAAATCCCATAAGTAATGTGAATTCCA
GAATTCAGTGAATCATCGAATCTTTGAACCCACCTTCCCCTCCTTCCGTATTCCTTCCGACCATCCCTGTT
TGAGTATCATGAAATTCTCAAACCCAATCTTTTTATTTTATTTAAAAACGTTGACTTTCGACTTCGACG
TCTTTTCCAGATGTTACAATCCTGCTCCTCTTAAATCCATTAGCTCCGATCTCAGTATATGACTTCGTTCC
TACTCCCCCGTGATAAGTAAAAACCTCCCTGACGACATTTTGTTTTATTTAGCAAAGTCCCCACCGTGT
AATCCAGATGAACCCCTTCTAATCGTCCATTAGCTTCCGACAAGACTTTCGTTTTTACAAGTCTTCTACTA
TATGATCTGATCTCAAATCAGG.

The ITS sequence of the Mandya isolate matched with that of S.hydrophilum reference isolates with Seq ID KX181457 and EU152863 with 100% identity .

The phylogenetic analyses of the ribosomal internal transcribed spacer (ITS) sequences of the isolates available in the NCBI database showed to be identical (99-100%) similarity grouped the isolates into three clades (Fig. 3). The I clade consist of the isolate MH393598.1, II clade with two isolates KX181457 and MH3670753 and third clade five isolates *viz*., KT362098, EU152863, LC317780, LC177545 and FJ595945.

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