

First report of *Ceratorhiza oryzae-sativae*, a fungus naturally causing aggregate sheath spot disease of rice in Manipur

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ABSTRACT

Rice is cultivated and consumed worldwide being a cereal crop of chief importance. There are numerous soil-borne fungi causing diseases of huge economic losses to production of rice. A survey of soil-borne fungi of rice was conducted in different valley districts of Manipur, and symptoms appearing as lesions, water-soaked spots near the waterline which often coalesces, forming concentric lesion bands were spotted. Among the collected symptomatic samples, a fungus, *Ceratorhiza oryzae - sativae* was identified. An internal transcribed sequence of the isolated fungus was generated and an NCBI accession number, MH255604 acquired. This disease might become severe in near future in all rice-growing fields of the state, and identification of this pathogen shall be essential for developing the management strategies.

Key words : Rice, Soil-borne fungi, *Ceratorhiza oryzae – sativae*, Aggregate sheath spot

Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop cultivated and consumed worldwide. It is chief cereal crop of India and a staple food for many states including Manipur. Rice is widely cultivated in both hilly and valley districts of the state. There are numerous soil-borne fungi in rice which can cause economic losses to the production of rice. A survey of soil-borne fungi in rice was conducted in different valley districts of Manipur during September and October 2018, where different fields were covered in the valley districts like Imphal West, Imphal East, Thoubal, and Bishnupur of Manipur (Table 1). A typical symptoms of lesions, water-

soaked spots near the waterline, and oval-shaped lesions with a size of 0.5–4cm long and grey to straw-colored centers surrounded by a brown margin were observed (Fig.1) and more number of spots often coalesces, forming concentric lesion bands. The collected samples were brought into the laboratory of the Department of Plant Pathology, COA, CAU, Imphal, where the isolation of the causal organism was carried out. The representative samples collected from different fields were subjected to microscopic observation.

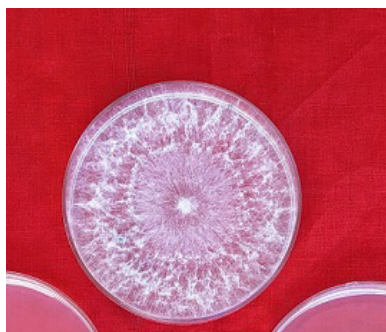
For isolation, diseased samples collected during surveys were taken and cut bits of the infected rice sheaths containing portions of healthy and diseased portions were surface sterilized by using 2% NaOCl

Table 1. Incidence of aggregate sheath spot diseases of rice in Manipur during 2018

Location	Disease incidence (%)	GPS Location		
		Latitude	Longitude	Altitude (ft.)
1. Imphal West	44.00	N-24°78.070	E-093°09.674	2746
2. Imphal East	49.50	N-24°45.827	E-093°03.138	2649
3. Bishnupur	49.68	N-24°25.927	E-093°43.074	2537
4. Thoubal	54.90	N- 24°3838	E-094°1482	4757

**Fig. 1.** Symptoms of the disease observed in the field

solution and rinsed using sterile distilled water thrice. The sterilized sheath portions were inoculated on PDA plates and kept inside the BOD chamber at a temperature of $28 \pm 1^\circ\text{C}$ and observed for mycelial growth. After 48hrs mycelial growth was observed, the mycelium was seen under a microscope and was found to produce septate type mycelium which is colorless or white when young and turns into dark brown on the advancement of growth (Fig. 2a). On the 5th day, sclerotial initiation was observed, after 10 days more sclerotia were formed all over the petri plate, they are mostly small, numerous, round to spherical, and commonly mustard seed shaped, white when young, and turns brown or black in older days. The size of sclerotia was in the range of 1.6-2.1 mm measured with the help of Stage and Ocular lens (Fig. 2b). Initially, a

**Fig 2a.** Mycelial growth observed on PDA medium**Fig 2b.** Formation of numerous sclerotia on PDA

morphological study showed that the pathogen's characters as *Rhizoctonia* spp. The fungal isolates were subjected to isolation of DNA using HiPurA™ Fungal DNA isolation Kit (Hi media, India) as per the manufacturer's protocol and amplified based on universal primer pairs ITS1 and ITS4, inter transcribed spacer region viz., ITS1 5'-TCCGTAGGTGAACCTGCGG - 3' & ITS4 5'-TCCTCCGCTTAT TGATATGC - 3' (White *et al.*, 1990). Approximately 700 bp nucleotides were amplified using PCR, which was confirmed by 1.2% agarose gel electrophoresis and purified DNA isolate sequenced further. The sequence had shown 96.18 percent identity with the Philippines isolate of *Ceratorhiza oryzae-sativae* (NCBI Accession no MH208454). The pathogen under study was identified as *Ceratorhiza oryzae-sativae* and the sequence has been submitted to NCBI and subsequently obtained accession number as MH255604. The pure culture of the identified pathogen, *Ceratorhiza oryzae-sativae* has been submitted to the Microbial Repository Centre (MRC) established at the Institute of Bio resources and Sustainable Development (IBSD), Manipur and subsequently obtained accession number as MRC 76071.

Further, Koch's postulate has proved the identity and confirmation of the pathogen, following the modified method of Aye *et al.*, (2009). Sterilized soil was taken in pots. Ten days old culture of pathogen



Fig 3. Symptoms of Aggregate sheath spot on artificially inoculated rice plants

developed on sterilized sorghum grains was mixed thoroughly with soil @ 5g inoculum in 1kg soil and 30 days old seedlings of CAU R-1 variety of rice were transplanted in the inoculated soil. Control plants were maintained separately without inoculum and inoculated seedlings were enclosed with polythene bags. After the symptom expression was observed (Fig. 3), re-isolation was made, and that was compared with the mother culture obtained from infected field-collected samples. Aggregate sheath spot of rice caused by *R. oryzae-sativae* was first described in Taiwan (Sawada, 1922). Since then, the disease has been reported in many Asian countries (Ou, 1985), Italy (De Carolis, 1973), India

(Mukherjee *et al.*, 1980). Isolates of *Rhizoctonia* spp. were obtained from rice in India during 2000–2003 and aggregate sheath spot of rice caused by *Rhizoctonia oryzae-sativae* has been distinguished from other *Rhizoctonia* spp. by amplified fragment length polymorphism (Taheri *et al.*, 2007). *Ceratorhiza oryzae-sativae* were identified as the cause of aggregate sheath spot of rice in Manipur. This disease might become in severe near future in almost all of the rice-growing fields of the valley districts of Manipur, and identification of this pathogen shall be essential for developing its management strategies.

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