Australasian Plant Dis. Notes (2015) 10: 10
DOI 10.1007/s13314-014-0155-9

First report of white mould caused by *Sclerotinia sclerotiorum* on jackfruit

Md Muzahid E Rahman · Tapan Kumar Dey · Delwar M. Hossain · Masanori Nonaka · Naoki Harada

Received: 30 April 2014 / Accepted: 15 December 2014 / Published online: 26 February 2015

Abstract Symptoms of white mould were first observed on jackfruit (*Artocarpus heterophyllus*) in Rangpur, Bangladesh, during February 2012. Fluffy, white mycelia developed on the fruit surface, along with large irregular black sclerotia. Morphological characteristics and the internal transcribed spacer sequences of ribosomal DNA identified the fungus as *Sclerotinia sclerotiorum*.

Keywords *Sclerotinia sclerotiorum* · Jackfruit · Bangladesh · Internal transcribed spacer (ITS) region

In Bangladesh, jackfruit (*Artocarpus heterophyllus*) ranks third in area and production next to mango and banana (BBS 2011). *Sclerotinia sclerotiorum* is one of the most non-specific, omnivorous, and necrotrophic phytopathogens (Purdy 1979) that was found to attack more than 400 different plant species worldwide (Boland and Hall 1994; Bolton et al. 2005). In Bangladesh, *S. sclerotiorum* was first recorded on mustard in 2008 (Hossain et al. 2008), then on chilli, aubergine, and cabbage (Dey et al. 2008). A new symptom that appeared as white mould was observed in a year-round jackfruit orchard in a colder part of the country at Rangpur (latitude: 25° 44′ N and longitude: 89° 14′ E), Bangladesh, during February 2012. The altitude of infection was up to 44 m above sea level. The average night temperature ranged from 15 to 25 °C during the period of February 2012 with foggy nights. Despite the application of different disease control methods, including fungicides, the disease continued to progress because the causal agent of the disease was not known. Moreover, the number of infected fruit increased rapidly after the infection initiated.

Symptoms began as a dark brown, water-soaked lesion that expanded rapidly on the fruit rind while rotting fleshy tissue internally. Lesions with necrotic tissues subsequently developed patches of fluffy white mycelia surrounded by a dark brown interaction zone on fruit surfaces (Fig. 1). Large amounts of sclerotia were produced inside the external flesh and outside the rind of infected fruits (Fig. 2).

Diseased fruit samples were collected from different plants in the infected orchard and preserved at the Plant Pathology Division, Bangladesh Agricultural Research Institute, for further study. Repeated isolations from the infected fruit were made on potato dextrose agar (PDA, 39 g/L; Himedia, India) after surface sterilisation with 70 % ethyl alcohol for 60 s followed by three consecutive rinses with sterile distilled water for 1 min. Whitish cultures developed with septate and hyaline mycelia and elliptical to globose or irregular-shaped sclerotia on the PDA medium (Fig. 3). Two isolates, JBdSs1 and JBdSs2, were obtained from separate samples. Typical apothecia formed from sclerotia after conditioning with alternating deep-freezing and normal temperature treatments for 3 consecutive days followed by 8 weeks of incubation on moist sand in a petri dish (Fig. 4). The isolates JBdSs1 and JBdSs2 were deposited to the National Institute of Agrobiological Sciences (NIAS) Genebank under the accession number of MAFF244849 and MAFF244850, respectively.

M. M. E. Rahman · T. K. Dey
Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh

M. M. E. Rahman · D. M. Hossain
Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

M. M. E. Rahman
Graduate School of Science and Technology, Niigata University, Niigata 950-2181, Japan

M. Nonaka · N. Harada (✉)
Institute of Science and Technology, Niigata University, Niigata 950-2181, Japan
e-mail: naharada@agr.niigata-u.ac.jp

Fig. 1 White mould symptoms on the fruit surface of jackfruit

Fig. 2 Sclerotia formation inside the flesh of naturally infected fruit

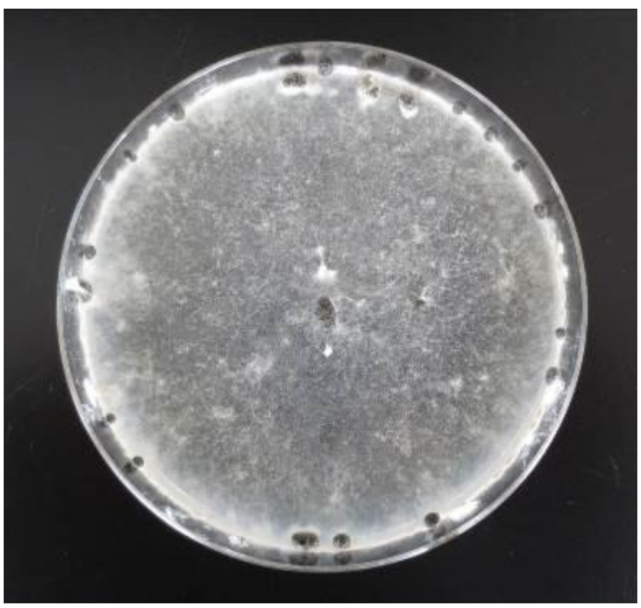

Fig. 3 Mycelial growth and sclerotia formation on a potato dextrose agar plate

Fig. 4 Apothecia developed from sclerotia after carpogenic germination on moist sand in a petri dish

For molecular characterisation, genomic DNA was extracted from fungal colonies of two isolates growing on PDA using the ISOPLANT kit (Nippon Gene, Tokyo, Japan). Internal transcribed spacers (ITS) of the ribosomal DNA of two isolates were amplified with the KOD FX Neo kit (Toyobo, Osaka, Japan) using the ITS4 and ITS5 primers (White et al. 1990) according to the manufacturer's protocol. Forward and reverse nucleotide sequences were determined from FASMAC (Kanagawa, Japan), and were edited and aligned using BioEdit 7.0.9®. DNA sequences of the ITS region of two isolates revealed 100 % identity to publicly available sequences of *S. sclerotiorum* (e.g. GenBank JQ618848,

Fig. 5 Whitish fluffy mycelia developed from the four inoculated points on the fruit rind after 7 days of incubation in a growth chamber during a pathogenicity test

HQ833450, and EF091809). The nucleotide sequences that were generated in this study from isolates JBdSs1 and JBdSs2 were assigned as GenBank accession AB898681 and AB898682, respectively.

To fulfill Koch's postulates and confirm its pathogenesis, detached fresh and healthy fruit were used. Unripe whole fruit (around 4 kg) were washed thoroughly in tap water, surface sterilised with 70 % ethyl alcohol for 60 s, immediately rinsed in sterile water, gently wrapped in sterile paper towel, and air dried inside a clean bench. An agar segment (1 cm^2) of a 5-day-old culture of *S. sclerotiorum* from PDA plates was placed on the rind surface. Mycelial blocks were inoculated separately in four places on each jackfruit. Three replications were made, and two fruit were tested in each replication. Altogether, eight points of inoculation were made per replication. A control treatment was maintained using a PDA block without the fungul culture. Fruit that were inoculated with *S. sclerotiorum* and un-inoculated fruit were kept for 15 days inside a growth chamber (MLR-351H, Sanyo, Japan), maintained at 20 °C with 95 % relative humidity and a 12-hour alternating photoperiod. Whitish fluffy mycelia developed on the inoculated point within 7 days, and mycelia covered an area of around 3 to 5 cm of the fruit surface (Fig. 5). After 14 days, up to 50 % of the inoculated side of all of the inoculated jackfruit was rotten.

To our knowledge, this is the first report of white mould disease on jackfruit caused by *S. sclerotiorum*. Through orchard and in vitro observation of the disease development, it was concluded that year-round jackfruit plants that yield fruit in winter are highly susceptible to *S. sclerotiorum*.

Acknowledgements The authors would like to thank Suchana Rani Sarker and M Hamim Reza from Bangladesh Agricultural Research Institute for their assistance in collecting samples.

References

BBS (2011) Year book of agricultural statistics of Bangladesh, 2010. Bangladesh Bureau of Statistics, Ministry of Planning. Govt of Bangladesh, Dhaka, Bangladesh

Boland GJ, Hall R (1994) Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian J of Plant Pathol 16:93–100

Bolton MD, Thomma BPHJ, Nelson BD (2005) *Sclerotinia sclerotior*um (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Mol Plant Pathol 7(1):1–16. doi:10.1111/J.1364-3703.2005.003 16.X

Dey TK, Hossain MS, Walliwallah H, Islam K, Islam S, Hoque E (2008) *Sclerotinia sclerotiorum*: An emerging threat for crop production. BSPC, BARI, Bangladesh, p 12

Hossain MD, Rahman MME, Islam MM, Rahman MZ (2008) White rot, a new disease of mustard in Bangladesh. Bangladesh J Plant Pathol 24:81–82

Purdy LH (1979) *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution and impact. Phytopathology 69:875–880

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols. a guide to methods and applications. Academic Press, San Diego, pp 315–322