FIRST REPORT

First report of twig blight of green pea caused by *Choanephora infundibulifera* in India

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ABSTRACT: The present report describes *Choanephora infundibulifera* as causal pathogen of twig blight of *Pisum sativum* recorded from the Gangetic alluvial region of West Bengal, India. Severe disease infestation with the initiation of shoot apical meristem, leaf and blossom blight was observed. The monsoon season (June-September) with optimum temperature (28-30°C) and high moisture (80-90%) level was found to enhance the disease progress. The pathogen was identified based on its cultural, morphological, and the internal transcribed spacer (ITS) - r DNA sequence analysis. To our understanding, this pathogen is being described for the first time as causal agent of twig blight disease of pea in India.

Keywords: Fungal pathogen, India, ITS, *Pisum sativum*, twig blight

Pisum sativum L. [Fabaceae] is an annual herbaceous leguminous vegetable widely cultivated in almost all the states of India. The routine survey is conducted in every year in the Gangetic alluvial region of West Bengal. During November 2015, twig blight in *Pisum sativum*, was noticed and recorded for the first time from Nadia, West Bengal. Disease incidence recorded ranged from 5-25%. Primary symptoms were deep to light green, gradually become brownish and appear as water-soaked lesions on infected parts. Emerging multisporous sporangia form cushion like mat or caterpillar's hairy appearance, with black headed sporangium on sporangiophores (Fig. 1). Favorable temperatures (28±2°C) coupled with high humidity (80-90%) played a vital role in triggering the emergence of this disease.

The fungus was isolated from lesions producing white to pale yellowish brown mycelia with cottony projections of multisporous sporangia scattered host surface on potato dextrose agar (PDA) (Saroj *et al.*, 2012). Both monosporous and multisporous sporangia were observed under both field and cultural conditions. The fungal colonies on PDA were cottony white in color with pale yellow pigmentation observed on PDA. Based on literature pigmentation appear probably due to β -carotene synthesis (Barnett *et al.*, 1956).

Morphological studies revealed that monosporous sporangia were elliptic or ovoid, 5.5-16.5 × 8-25 μ m. Sporangium sub-globose, 30-90 μ m. Sporangiospores elliptic, fusiform, or ovoid, 6-14.5 × 8-25 μ m (Fig. 2).

Fig. 1. (A) Water soaked brownish spot on stem; (B) Emerging sporangia from stem and leaf surface; (C) Twig blight

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Fig. 2. (A) Culture on PDA; (B) Single sporangia; (C) Sporangiospores

Morphological structures are within the given range of *C. infundibulifera* database (Kirk, 1984). Each end of the bipolar sporangiospores attached with three or more hyaline appendages. Sporangiospores were elliptic, fusiform or ovoid in shape, light brown or dark brown in color and released singular triads or tetrads forms in basipetal successive order at the time of spore dispersal. Zygosporangium formation was not observed in this study (Kwon *et al.*, 2005). The optimum growth temperature was recorded about $28 \pm 2^\circ\text{C}$ on PDA. The growth rate of the isolate P.S-16 was very first encroached whole Petri dish (9 cm diam.) within 48 h. Based on the overall morphological characteristic, the fungus was placed under *Choanephora infundibulifera* (Curr.) D.D. Cunn.

(Choanephoraceae: Mucorales). The herbarium specimen of infected plant and culture of the isolate P.S-16 was deposited in HCIO (Herbarium Cryptogamae Indiae Orientalis, Indian Agricultural Research Institute, New Delhi, India) and ITCC (Indian Type Culture Collection, Indian Agricultural Research Institute, New Delhi, India) with the accession no. ITCC no. 7977 and HCIO no. 52048.

The molecular identification of fungus was performed using sequencing of ITS regions, reason being the large ITS data collection for fungi, extremely variable nature within highly conserved ribosomal coding sequences and high popularity in studies dealing with

Fig. 3. Phylogram recovered from a maximum likelihood search in RAxML with an alignment of ITS sequences

fungal identification and phylogenetic relationships (Boysen *et al.*, 1996). ITS region of nuclear rDNA was amplified using ITS-1 and ITS-4 primer pairs (White *et al.*, 1990). BLAST search revealed that the isolate had 100% identity with *C. infundibulifera* (Genbank accession numbers: KU316934, KU877802, KM200034, KJ461159 and JN943006). The nucleotide sequence was deposited in Genbank under accession no. KX462161. The phylogenetic analysis was done (Fig. 3) by using MEGA6 (Tamura *et al.*, 2013).

The pathogenicity was established by using two techniques. In the first technique, 45 days old potted *Pisum* seedlings grown in growth chamber and detached twigs (6 cm) in Petri plates were inoculated with pre-grown fungal mycelial discs. A single disc was attached to 2-3 cm below pre-wounded shoot apical meristem of the seedlings, coiled with thin film of moist absorbent cotton, covered with polythene bags and incubated at $28\pm 2^{\circ}\text{C}$ and 80-90% relative humidity in a growth chamber. Detached twigs were inoculated with mycelial disc in the notched stem portion, coiled with thin film of moist absorbent cotton, put inside a Petri plate sandwiched with moist blotting paper and incubated in BOD incubator at $28\pm 2^{\circ}\text{C}$. In the second technique, 45 days old potted *Pisum* seedlings and detached twigs (6 cm) in Petri plates were inoculated with spraying spore suspension (1×10^6 spores/ml in sterile distilled water) and incubated as stated above. Pathogenicity tests showed the similar symptoms as in field condition after 4-5 days after inoculation (DAI) for mycelial disc-inoculation or 7-8 DAI with spore suspension method. Based on the symptomatology, isolate P.S-16 was identified as *C. infundibulifera*. Previously, plant pathogenic zygomycetes were considered as weak parasites (Agrios, 2005), but it appears that some zygomycetous fungi evolved their behaviour in such a way that their complex adoptability prefers a new host

as a substitution of their trend line. To our knowledge, this is the first report of twig blight disease of *Pisum sativum* caused by *Choanephora infundibulifera* in India.

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