Leaf rot disease of cauliflower caused by *Choanephora cucurbitarum* in India

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Received: 31 December 2014 / Accepted: 26 September 2016 / Published online: 7 October 2016
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Abstract Leaf rot of cauliflower caused by *Choanephora cucurbitarum* is reported for the first time in India. Koch's postulates were fulfilled for the first time for this fungus-host association demonstrating that *C. cucurbitarum*, known to be the pathogen behind soft rot on numerous hosts is also a pathogen of cauliflower.

Keywords *Brassica oleracea* · Mucorales · Soft rot · Vegetable diseases · Zygomycota

Choanephora cucurbitarum (Mucorales) is an important plant pathogen that causes soft rot of floral parts and fruits of many plants, including summer squash, pumpkin, pepper and okra (Agrios 1997). Cauliflower (*Brassica oleracea*) is an important crop in India and any diseases found on this host are of potential agricultural and economic relevance.

In September 2012, 2013 and 2014, leaves of an early variety of cauliflower (cv Early Kuary) bearing typical soft rot symptoms, accompanied by superficial fungal growth

were collected at Palarikhurd Village, Sonipat (28°59'26.53" N; 77°00'57.30" E; 249 m a.s.l.), Haryana, India. Disease symptoms were observed and photographed in the field. Samples were taken for later examination in the laboratory. Fragments of infected leaves bearing pin head-like fungal growth were surface sterilized by dipping in 1 % sodium hypochlorite for 1 min and rinsing once with sterilized distilled water and aseptically transferred to plates containing PDA (potato dextrose-agar) and left at room temperature for 48 h. Homogeneous cultures were obtained and one was selected for further study and deposited in the Indian Type Culture Collection (ITCC 7521).

Slides were prepared by mounting a colony fragment in lactophenol. The fungal structures were observed under a microscope (Olympus, Magnus MLXi). The morphological and cultural characters of the fungus were recorded and compared with published descriptions of soft rot-fungi (Kwon et al. 2001; Kagiwada et al. 2010). Profuse and rapidly growing white mycelium was formed on PDA after 36 h. Sporangiohores bearing apical sporangiola were formed. Sporangia were sub-globose (35 – 85 µm diam) and monosporous sporangiola were elliptic, fusiform or ovoid (8–13 × 11–22 µm) (Fig. 1c). Sporangiospores were elliptic, fusiform or ovoid (7–10.5 × 10–27 µm), brown, with or without three or more thin appendages at both ends (Fig. 1d). This morphology is typical of *C. cucurbitarum* as described by Kwon et al. (2001) and Kagiwada et al. (2010).

Identity of the fungus involved on cauliflower leaf rot was further investigated by comparison of ITS and TEF sequences of ITCC 7521 with GenBank data (White et al. 1990).

Genomic DNA was extracted from the pure culture of *C. cucurbitarum* through the cetyl trimethyl ammonium bromide (CTAB) method with slight modifications (Karthikeyan et al. 2010). DNA extracted by adding chloroform: isoamyl alcohol in the ratio of 24:1 centrifuged at 12,000 rpm/10 min

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Fig. 1 Symptoms and signs of *Choanephora* rot on cauliflower and morphology of the causal agent *Choanephora cucurbitarum* **a** Symptom of leaf rot of cauliflower in field; **b** Numerous monosporous sporangia (at 10 x 100X); **c** Fusiform sporangiospores with appendages (at 10x100X); **d** Cauliflower seedlings showing typical symptoms of leaf and bud rotting

repeated this step twice, suspended with 150 μ l of TE buffer (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0). RNA was digested by adding 2 μ l of RNase A and incubated at 37 °C for 1 h and stored -20 °C for further use.

The internal transcribed spacer (ITS) region was amplified with universal primers ITS1 and ITS4 (White et al. 1990). The reaction mixtures were prepared in a total volume of 100 μ l with a final concentration of 50 ng DNA, 10 μ l of 10 $\times$ assay buffer, 1.5 μ M MgCl₂, 10 μ M dNTP mix, 10 pM of each primer, and 2.5 U of Taq DNA polymerase (Bangalore Genei, India) and the volume was made up to 100 μ L with sterile double distilled water. The PCR was performed with a PCR Thermal Cycler MP (Bioer, Japan) according to the following program sequence: initial denaturation 94 °C for 3 min, followed by 35 cycles of 1 min at 94 °C, 30 s at 60 °C and 1 min at 72 °C with a final extension step for 3 min at 72 °C. After thermal cycling, the amplified product was run on a 1.2 % agarose gel, stained with ethidium bromide, The gel was visualized under UV radiation in a gel documentation system (Biorad, USA) and digitally photographed (Canon, Tokyo, Japan).

Translation elongation factor (TEF) was amplified with specific primers EF1-645F and EF1-1190R (Fiers et al. 2011). The reaction mixture prepared to the total volume of 50 μ l with final concentration of 75 ng of DNA, 5 μ l of 10X Taq buffer, 1.5 μ M MgCl₂, 8 μ M dNTP mix, 10 pM of each primer, and 1.5 U of Taq DNA polymerase (Bangalore Genei, India) and the volume was made up to 50 μ L with sterile

double distilled water. The PCR was performed with aPCR Thermal Cycler MP (Bioer, Japan) according to the following program sequence: initial denaturation 94 °C for 2 min, followed by 30 cycles of 1 min at 94 °C, 30 s at 56 °C and 1 min at 72 °C with a final extension step for 10 min at 72 °C. The amplified product was electrophoresed and digitally photographed as cited above.

The amplified products of ITS and TEF were partially sequenced at Amnion DNA sequencer (Bengaluru, India). The sequences were deposited in NCBI GenBank under the following accession numbers (for ITS - KJ817815, for TEF- KM214205). A BLAST search in GenBank revealed 99 % NT identity with *C. cucurbitarum* as per the comparison with the accession numbers KF724997 for ITS and AF157239.1 for TEF. Identity of the isolate ITCC 7521 was therefore confirmed as *C. cucurbitarum*.

Healthy seedling of cauliflower (cv Early Kuary) were planted individually in each of ten plastic pots containing containing sterilized sandy loam soil. When the plants were thirty days-old they were inoculated with 5 mm diam, sporulating culture discs obtained from 5 days old ITCC 7521 colonies formed on PDA. Discs were placed on leaf margins (two leaves/plant) and tender buds. The pots were then covered with an internally moistened plastic bag and kept inside an incubator (Ferrotek) adjusted to 25 °C. The plastic bags were removed after three days. As a control, PDA disks with no fungus were deposited on leaves of two separate plants and these were treated as described above. Typical soft rot

symptoms appeared after 5 to 7 days of inoculation, firstly at the margins of younger leaves as water soaked areas, followed by inward rolling and rapid rotting of the infected leaves that became black (Fig. 1f). Later (7 days after inoculation) the infected areas were covered with pale greyish brown fungal colonies (Fig. 1g). This is the first record of *C. cucurbitarum* causing leaf rot of cauliflower in India and the first time the pathogenicity of this fungus to cauliflower was demonstrated.

References

- Agrios GN (1997) Plant pathology. Academic Press, USA
- Fiers M, Edel-Hermann V, Héraud C, Gautheron N, Chatot C, Le Hingrat Y, Bouček-Mechiche K, Steinberg C (2011) Genetic diversity of *Rhizoctonia solani* associated with potato tubers in France. *Mycologia* 103:1230–1244
- Kagiwada S, Kayano Y, Hoshi H, Kawanishi T, Oshima K, Hamamoto H, Horei H, Namba S (2010) First report of *Choanephora* rot of ice plant (*Mesembryanthemum crystallinum*) caused by *Choanephora cucurbitarum* in Japan. *J Gen Plant Pathol* 76:345–347
- Karthikeyan V, Patharajan S, Palani P, Spadaro D, Gullino ML, Garibaldi (2010) Modified simple protocol for efficient fungal DNA extraction highly suitable for PCR based methods. *Global J Mol Sci* 5:37–42
- Kwon JH, Shen SS, Park CS (2001) Pod rot of cow pea (*Vigna sinensis*) caused by *Choanephora cucurbitarum*. *Plant Pathol J* 17:354–356
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR protocols: A guide to methods and applications, MA Innis, DH Gelfand, JJ Shinsky, TJ White (ed) Academic Press, San Diego