Bipolaris coicis causing adlay leaf blight in Iran

A. Ahmadpour · A. Pordel · Z. Heidarian ·
M. Javan-Nikkhah

Received: 20 July 2013 / Accepted: 2 October 2013 / Published online: 26 October 2013
© Australasian Plant Pathology Society Inc. 2013

Abstract During the summer of 2012, some leaves of *Coix lacryma-jobi* (adlay or Job's tears) with leaf spot symptoms were collected from surrounding rice fields of Amol, north Iran. Four single spore isolates were obtained from diseased adlay leaves and the pathogenicity of each isolate to adlay plants was confirmed by inoculation tests based on Koch's postulates. The pathogen was identified as *Bipolaris coicis*, based on both morphological characteristics and sequencing of the internal transcribed spacer region of the rDNA. This is the first report of *Bipolaris coicis* causing leaf blight on adlay in Iran.

Keywords Gramineous plants · Pathogen identification · Morphology · Pathogenicity

Adlay or Job's tears (*Coix lacryma-jobi*) is a tall grain-bearing tropical plant of the family Poaceae (grass family) native to southeast Asia. Seeds of adlay perennial grasses are used ornamentally and as a medicinal and cereal food in many countries (Jain and Banerjee 1974; Arora 1977; Chang and Hwang 2002). In Iran, this is a weed in rice fields and also the overwintering host of rice stem borer (*Chilo suppressalis*). *Bipolaris coicis* (teleomorph:

Cochliobolus nisikadoi), the cause of leaf blight of adlay, is one of the major limiting disease and destructive foliar pathogens in cultivating areas of adlay (Chang and Hwang 2002).

During the summer of 2012, some leaves of adlay with leaf spot symptoms were collected from surrounding rice fields of Amol, north Iran. Leaf spot symptoms were large, elongated and pale brown to brown with yellow margins (Fig. 1a). A single spore produced on the surface-sterilized leaf was transferred to water-agar and then fungal colonies were produced by hyphal tip method on potato dextrose agar (PDA) culture medium. A subculture was transferred to tap water agar with autoclaved wheat straw (TWA+wheat straw) plates for 10–14 days (Sivanesan 1987). Four isolates (Cb1, Cb2, Cb3 and Cb4) of *Bipolaris* sp. were isolated from lesions on symptomatic leaves. Measurements and microphotographs were taken from slide mounts in lactophenol using an Olympus light microscope (model BH2). The fungal colony was gray on potato dextrose agar (PDA). Conidiophores were single to fasciculate in small groups, cylindrical, brown, 600–1,200 µm long (Fig. 2a, b). Conidia straight, occasionally curved, oval, obclavate, pale brown to brown, 3–4 distoseptate, 25–40×9–16 µm (Fig. 2b–e). Based on morphological characteristics, the isolates were further identified as *Bipolaris coicis* (Sivanesan 1987). Dried culture vouchers have been deposited in the herbarium in the Department of Plant Protection, Faculty of Agricultural Sciences & Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (TUPP).

Genomic DNA was extracted using the method of Liu et al. (2000). One isolate (Cb1) was subjected to PCR amplification of the internal transcribed spacer (ITS) 1 and ITS2 regions flanking the 5.8S rRNA gene. This was

A. Ahmadpour (✉) · A. Pordel · M. Javan-Nikkhah
Department of Plant Protection, University College of Agriculture
and Natural Resources, University of Tehran, Karaj, Iran
e-mail: ahmadpour.abdollah@gmail.com

M. Javan-Nikkhah
e-mail: jnikkhah@ut.ac.ir

Z. Heidarian
Department of Plant Protection, Faculty of Agriculture, University of
Tabriz, Tabriz, Iran

Fig. 1 Symptoms of leaf spot caused by *Bipolaris coicis* on adlay. **a** leaf spot symptoms on naturally infected adlay in field. **b** leaf spot symptoms on infected adlay under greenhouse conditions 10 days after inoculation

carried out with universal primers ITS1 and ITS4 according to the published protocol (White et al. 1990). The reaction mixture and PCR conditions were the same as described by Ahmadpour et al. (2012). The sequence was deposited into GenBank (KC315945). The ITS sequence comparison showed 98 % identity to *B. coicis* type strain CBS 192.29 (GenBank accession AF081447). According to morphological and molecular analysis, the isolates were identified as *B. coicis*.

For the pathogenicity test, adlay plants were sown in plastic pots containing a sterilised soil in the greenhouse. Four isolates of *B. coicis* were cultured on TWA+wheat straw at 25 °C for 10 days. The conidial suspensions of *B. coicis* were adjusted to 10^5 conidia/ml using a hemacytometer (Chang and Hwang 2003). The pathogenicity tests were conducted using three replicates for each isolate and two plants for each replicate. Inoculated plants were placed in the greenhouse at 20–

25 °C and 75–85 % RH along with control plants that had been sprayed with sterile, distilled water. Plants were covered with plastic bags for 48 h to maintain high humidity. Inoculated plants were inspected daily for disease symptom development for 10 days after inoculation. Leaf lesions were visible on all inoculated plants after 10 days (Fig. 1b), but symptoms were not observed on control plants. The spots were pale brown to brown with yellow margins (Fig. 1b). The fungus was reisolated from leaf lesions on inoculated plants and morphologically identified, but not from leaves of control plants, confirming Koch's postulates.

Bipolaris coicis has previously been reported on some monocotyledonous plants including corn, wheat, barley and tiger grass (*Thysanolaena maxima*) (Sivanesan 1987; Cho and Shin 2004; Manamgoda et al. 2012). To our knowledge, this is the first record of leaf spot disease on adlay by *B. coicis* in Iran.

Fig. 2 *Bipolaris coicis*. **a–b** conidiophores. **c–e** conidia. Bars (**a**) = 100 μ m, (**b–e**) = 10 μ m

Acknowledgments The authors would like to thank the University of Tehran for financial support.

References

- Ahmadpour A, Heidarian Z, Donyadoost-Chelan M, Javan-Nikkhah M, Tsukiboshi T (2012) A new species of *Bipolaris* from Iran. *Mycotaxon* 120:301–307
- Arora RK (1977) Job's tears (*Coix lacryma-jobi*)—a minor food and fodder crop from northeastern India. *Econ Bot* 31:358–366
- Chang SW, Hwang BK (2002) Relationship of host genotype to *Bipolaris* leaf blight severities and yield components of adlay. *Plant Dis* 86:774–779
- Chang SW, Hwang BK (2003) Evaluation of virulence to adlays of Korean isolates of *Bipolaris coicis* using a disease rating scale. *Plant Dis* 87:726–731
- Cho WD, Shin HD (2004) List of plant diseases in Korea, 4th edn. Korean Society of Plant Pathology, 779 pp
- Jain SK, Banerjee DK (1974) Preliminary observations on the ethnobotany of the genus *Coix*. *Econ Bot* 28:38–42
- Liu D, Coloe S, Baird R, Pederson J (2000) Rapid mini-preparation of fungal DNA for PCR. *J Clin Microbiol* 38:471
- Manamgoda DS, Cai L, McKenzie EHC, Crous PW, Madrid H, Chukeatirote E, Shivas RG, Tan YP, Hyde KD (2012) A phylogenetic and taxonomic re-evaluation of the *Bipolaris*-*Cochliobolus*-*Curvularia* complex. *Fungal Divers* 56:131–144
- Sivanesan A (1987) Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. *Mycological Papers No. 158*, CAB International, Wallingford, UK
- 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, San Diego, pp 315–322