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Short report

First report of *Colletotrichum fructicola* as the causal agent of anthracnose on common bean and cowpea

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Common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) are the two most important commercial crops in Northern Iran. Anthracnose makes considerable economic losses on the crops worldwide.

During two consecutive years 2013 and 2014, distinct anthracnose symptoms were observed on leaves and pods of common bean and cowpea in Mazandaran, Guilan and Zanjan provinces, Iran. Fungal isolates were isolated on 2% Water Agar (WA) and standard Potato Dextrose Agar (PDA) amended with chloramphenicol (50 mg/L). In order to identify the fungal isolates, they were transferred to PDA, Oatmeal Agar (OA) and Synthetic Nutrient-poor Agar (SNA) media. Cultures were incubated at 20 °C under near-UV light with a 12 h photoperiod for 14 days. To enhance sporulation, sterile paper and double-autoclaved stems of *Anthriscus sylvestris* (cow parsley) were placed onto the surface of SNA medium. Morphological characters were recorded after 10 days (Damm et al. 2009).

A total number of 40 isolates were isolated from infected plants. Colonies on OA were white at first, becoming grey to dark grey at the center with age, in reverse greyish green with white halo max; colony diameter was about 68 mm in 7 days (> 90 mm in 10 d). Aerial mycelia were pale grey, dense, cottony, without visible conidial masses. Sclerotia were absent; setae rare; conidia one-cellular, smooth-walled, hyaline, cylindrical with obtuse to slightly rounded ends, sometimes oblong, (10.4)12.21–13.6(–15.92) × 3.7–4 (–4.74) µm, mean ± SD = 13.04 ± 2.26 × 4.11 ± 0.48 µm, L/W ratio = 3.17 (fig. 1); ascomata perithecia, light brown to brown, globose to subglobose, 125–560 × 100–280 µm on culture media, 130–270 × 60–110 µm on leaf,; asci unitunicate, thin-walled, 6–8 spored, clavate or cylindrical, 24–83 × 5–10 µm,; ascospores one-celled, hyaline, large, slightly curved to curved with obtuse to slightly rounded ends, (11.33–) 12.79–13.91(–15.12) × (3.58–)4.04–4.75(–5.25) µm, mean ± SD = 13.29 ± 1.52 × 4.40 ± 0.74 µm, L/W ratio = 3.02,; appressoria mostly formed from mycelia, brown to dark brown, ovoid, clavate and slightly irregular to irregular in shape and often becoming complex with age, 8–18 × 5–15 µm, mean ± SD = 12.4 ± 2.54 × 8 ± 2 µm, L/W ratio = 1.55.

Based on these morphological characters, the isolates were tentatively identified as a taxon within *Colletotrichum gloeosporioides* species complex (Weir et al. 2012).

The *β-tubulin* (*TUB2*) and *Glutamine synthetase* genes were then amplified, using the primers T1/Bt2b and GSF1/GSR1 which were sequenced locally (O'Donnell and Cigelnik 1997; Glass and Donaldson 1995; Stephenson et al. 1997) and deposited under GenBank accession No. KT189676 and No. KT189671, respectively. A sequence similarity search was performed using BLASTn (Altschul et al. 1990) algorithm available via GenBank, which confirmed the identification as *C. fructicola*.

Pathogenicity tests were carried out by spraying a conidial suspension (10^6 spores/ml) from a seven-day-old culture of the fungus on leaves and stems of common bean and cowpea seedlings (Balardin et al. 1997). Initially, seedlings were maintained at 25±5°C. Thereafter, seedlings were covered with plastic bags and transferred to a greenhouse. Anthracnose symptoms on the leaves were observed 4 days after inoculation on cowpea seedlings and 10 days after inoculation on common bean seedlings. On the living leaves, globose to ellipsoid to irregular leaf spots,

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with a brown center surrounded by a yellow margin were observed.

Symptoms on the cowpea seedlings were more severe than common bean. Seedlings from the control treatment remained symptomless. The fungus was re-isolated from the necrotic lesions, performing Koch's postulates (Fig. 1). *C. fructicola* isolates are biologically and geographically diverse. They have been isolated from *Pyrus pyrifolia* from Japan, *Limonium* from Israel, *Malus domestica* and *Fragaria ananassa* from the USA, *Persea americana* from Australia, *Ficus* from Germany, *Malus domestica* from Brazil, *Dioscorea* from Nigeria and *Theobroma* and *Tetragastris* from Panama (www.q-bank.eu). To our knowledge, this is the first report of occurrence of *C. fructicola* as the causal agent of anthracnose on common bean and cowpea plants in the world.

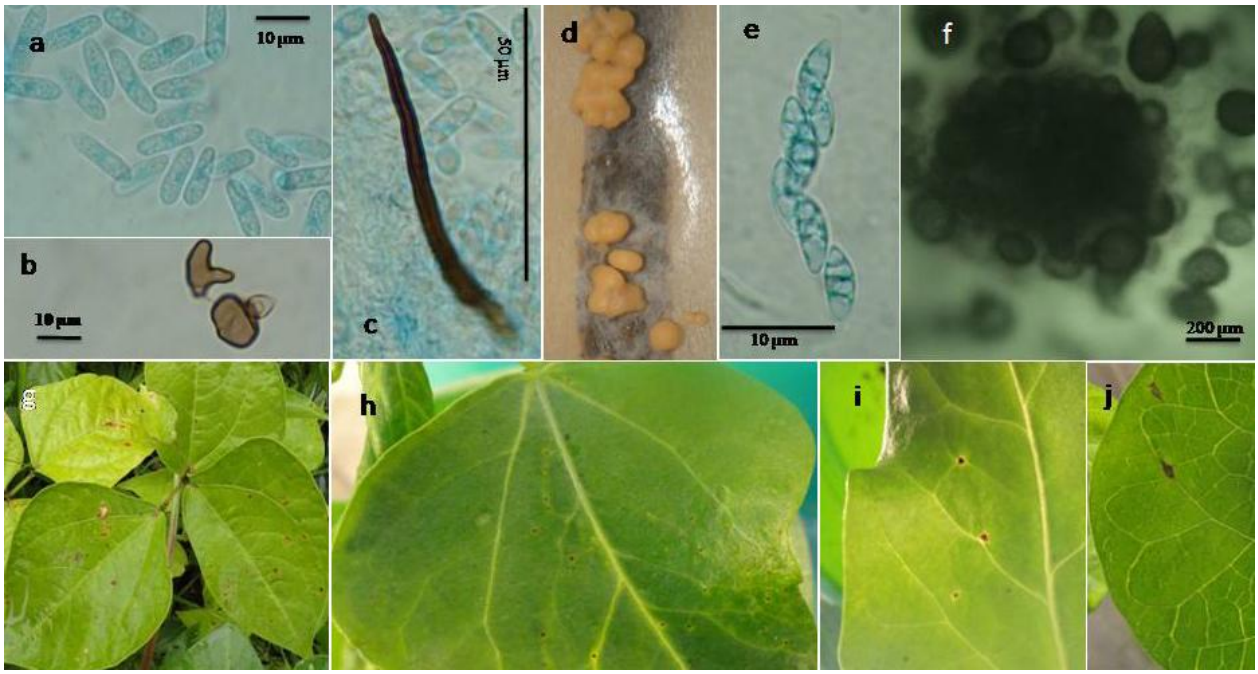

Fig. 1. *Colletotrichum fructicola.* **a**. Conidia, **b**. Appressoria, **c**. Setae, **d**. Conidiomata on *Anthriscus* stem, **e**. Asci, **f.** Perithecia on leaf spots on cowpea, 14 days after inoculation, **g.** Anthracnose symptoms on cowpea in field, **h.** Anthracnose symptoms on cowpea, 4 days after inoculation in greenhouse, **i.** Anthracnose symptoms on cowpea, 10 days after inoculation in greenhouse, **j.** Anthracnose symptoms on common bean, 10 days after inoculation in greenhouse.

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