New Disease Reports

First report of *Colletotrichum circinans* causing smudge on onion in Iran

S. Leylaie, D. Zafari* and Sh. Bagher Abadi

Department of Plant Protection, College of Agriculture, University of Bu Ali Sina, Hamadan, Iran

*E-mail: Zafari_d@yahoo.com

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In October and November 2012, white-scaled onions bulbs with necrotic symptoms were collected in the area under cultivation in Khuzestan province. Symptoms were dark spots frequently organised in concentric rings, forming patches of up to 1 cm in diameter (Fig. 1). These spots are made up of stromatic masses formed beneath the cuticle of the host, consisting of intertwining thick-walled mycelial threads in dark green to black stromata, usually only a fraction of a millimetre in diameter and a few to several hundred microns thick. The affected tissue was surface sterilised with sodium hypochlorite (0.8% chlorine) for 30 seconds and dried with sterile paper before small pieces were excised and aseptically transferred to potato dextrose agar (PDA) and water agar (WA) in petri dishes and incubated in the dark at ambient temperature (30 \pm 2°C). Isolates grown on PDA, produced acervuli with dark, almost black, 1-3 septate acicular setae having a bulbous base, measuring up to 290 x 8 μm (Fig. 2 A, B). Conidia were hyaline, unicellular, 16-25 x 3-5 µm, slightly falcate, guttulated (Fig. 1 C).

A small amount of mycelia was scraped from a seven-day-old culture and the internal spacer region (ITS) of the extracted fungal DNA was amplified with universal primers ITS1 and ITS4. The resulting sequence (509 bp) was submitted to the NCBI GenBank (isolate IRAN2185C, Accession No. KJ458968,). A blast search of ITS sequences revealed that this fungus was *Colletotrichum circinans* with 100% query coverage with KC790955 and AJ301955 and 99% query coverage with GQ369595. Diseased tissue was cut into 1-2 cm lengths, surface-sterilised, and the original pathogen from onion placed on water agar to recover the fungus. Three isolates of *C. circinans* were tested for pathogenicity to onion bulbs by artificial inoculation with conidial suspensions (1-2 x 10 conidia/ml) that were prepared from 20-day-old PDA cultures. Sterile distilled water was used as control. Plastic boxes containing the inoculated material were placed in an incubator at 26°C for five days. Disease ratings were made based on the degree of anthracnose symptoms induced five days after inoculation. The inoculation test was performed in three independent replicates. *C. circinans* was re-isolated from these lesions fulfilling Koch's postulates. *C. circinans* was previously recorded as *C. dematium* (Pers.) Grove f. *circinans* Arx, which is specific to *Allium* spp. (von Arx, 1970; von Arx, 1981). However, Sutton (1992) described *C. circinans* as a distinct species from *C. dematium*. The fungus causes smudge or leaf spot of *Allium* spp. (Farr *et al.*, 1989) and has been reported from Korea, Japan, China , Argentina, India, UK and most other regions of the world (Cho & Shin, 2004; Kiehr *et al.*, 2012). This the first report of *C. circinans* in Iran. A culture of *C. circinans* was deposited in the Iranian Research Institute of Plant Protection (IRAN2185C).

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Figure 1



Figure 2

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