New Disease Reports

First report of *Phaeoacremonium tuscanum* associated with grapevine decline disease in Iran

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Over the last few years, grapevine trunk diseases have been increasing in importance in Iran and several recent studies have shown that different pathogens contribute to such diseases in this country (Mohammadi *et al*., 2008; Gramaje *et al*., 2009; Mohammadi *et al*., 2009; Mohammadi, 2011). During August and September 2010, a field survey was carried out in Kohgiluyeh and Boirahmad province (south-western Iran) to study grapevine decline diseases. Samples were collected from symptom-bearing grapevine trunks and cordons showing yellowing, reduced growth, defoliation, small chlorotic leaves (Fig. 1) and internal black spots and wood discolouration in cross section (Fig. 2). Bark from each piece was removed and ten thin cross sections (2-3 mm thick) were cut from symptom-bearing tissue. These were immersed in 1.5% sodium hypochlorite solution for three minutes, washed three times with sterile distilled water and plated onto malt extract agar (MEA) supplemented with 100 mg/l streptomycin sulphate. Two isolates of a *Phaeoacremonium* sp., morphologically different from other *Phaeoacremonium* species previously reported in Iran, were isolated from 20-year-old grapevine rootstock (*Vitis vinifera* cv. Askari) originating from Yasuj (capital of Kohgiluyeh and Boirahmad province). Single conidial isolates were obtained and grown on potato dextrose agar (PDA), MEA and oatmeal agar (OA) media and incubated at 25°C for 8 to 16 days in the dark (Mostert *et al*., 2006). Colonies reached a radius of 7.1, 5.5 and 10.4 mm after 8 days incubation on MEA, PDA and OA respectively. Colonies were flat, pale brown to beige on MEA, flat, somewhat pale grey becoming whitish with age on PDA and flat and pale grey after 16 days on OA. Conidiophores were mostly short and usually unbranched, 15-37 (26.5) µm long, commonly ending in a single terminal phialide. Phialides were terminal or lateral and mostly monophialidic. Conidia were hyaline, cylindrical to ellipsoidal, 2.5-5.5 (3.9) µm long, and 1.2-1.9 (1.5) µm wide. Based on cultural and morphological characters, the isolates were identified as *Phaeoacremonium tuscanum* (Essakhi *et al*., 2008).

Additionally, identity of PTH1 isolate was confirmed by partial sequencing of the β-tubulin gene using primers T1 and Bt2b (GenBank Accession No. JN210897). The sequence of this isolate showed 100% similarity to a corresponding sequence from *Phaeoacremonium tuscanum* from Italy (U863458). Pathogenicity of both isolates was tested on two-month-old grapevine seedlings of cv. Askari by watering the roots with 25 ml of a conidial suspension (10^7 conidia/ml) harvested from 20-day-old cultures grown on MEA. Control plants were watered with 25 ml of sterile distilled water. Ten replicates were used for each isolate with an equal number of non-inoculated plants. All plants were grown under greenhouse conditions (25-30°C) and symptoms were checked periodically for two months. By the end of the experiment, inoculated seedlings showed chlorotic leaves, severe defoliation and wilting, while control plants remained symptomless. Koch´s postulates were confirmed by re-isolation of the fungus from internal tissues of the stems and crown of inoculated seedlings. This is the first report of *P. tuscanum* causing grapevine decline in Iran and outside of Italy.

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Figure 1

Figure 2

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