First report of *Neoscytalidium novaehollandiae* associated with stem canker and branch dieback of almond trees

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Abstract

In 2019, a new disease of stem canker with branches dieback was observed in several commercial orchards in Diyarbakır province, Turkey. The pathogen was identified as *Neoscytalidium novaehollandiae* based on morphological characteristics and phylogenetic analysis of both internal transcribed spacer and transcription elongation factor 1- α regions. Koch's postulates were confirmed by successful reisolation of *N. novaehollandiae* only from plants inoculated with the pathogen. This is thought to be the first report of *N. novaehollandiae* causing stem canker of almond trees.

Keywords Almond *Neoscytalidium novaehollandiae* stem canker

Almond (*Prunus dulcis*) is considered an economically important fruit crop worldwide. Turkey is the world's fifth largest almond producer with a production of 90.000 tons and a cultivated area of 34.050 ha (Faostat 2019). Almond orchards have continuously increased especially in the South East Anatolia region of Turkey. Trunk and canker diseases are a potential threats to almond production in Turkey. Some Botryosphaeriaceous fungi (*Neofusicoccum parvum* and *Diplodia* sp.) have been reported to cause wood canker and dieback symptoms on almond trees (Kayım et al. 2015).

In the summer of 2019, during a survey of diseased almond trees in commercial orchards in the localities Sur, Çermik, and Eğil of Diyarbakır province, symptoms of trunk diseases were observed, including yellowing and defoliation of leaves, stem canker, branch dieback, gummosis, vascular discoloration, and tree death (Fig. 1). To identify the causal agents, small pieces of necrotic tissues were collected from stem and branches of seven trees, surface disinfected with 2%NaOCl for 2 min, rinsed with sterile distilled water and plated onto potato dextrose agar (PDA) amended with 0.5 g L⁻¹ streptomycin sulfate. Plates were maintained at 25 °C in dark and growing colonies were examined for

cultural and morphological characteristics. All affected tissues consistently developed colonies with a white mycelium, becoming dark gray to black within a week. A total of 11 isolates were identified as *Neoscytalidium*-like based on morphological characteristics. Conidia were dark brown, thick-walled, cylindrical to oblong, 0 to 1 septate, 6.0 to 7.5 $\times$ 3.5 to 4.4 μ m ($n = 30$), formed both singly and in disarticulating arthric chains from the aerial mycelium. Pycnidia observed on pine needles in the culture were stromatic, semi-immersed, and black. Conidia were ellipsoidal to oval, apices rounded, initially hyaline, becoming sepia 0–1-septate or 2-septate, 11.3 $\times$ 4.5 μ m. The morphological characteristics of the isolates coincided with those of *Neoscytalidium novaehollandiae* (Phillips et al. 2013). Also, the identification of the causal fungi was confirmed by DNA sequencing analysis.

Genomic DNA of the representative *N. novaehollandiae* isolate An-2 was isolated using Qiagen plant tissue DNA extraction kit according to the manufacturer's instructions (Qiagen, USA). The internal transcribed spacer (ITS) nrDNA and translation elongation factor 1- α (EF1- α) gene regions were amplified using the primers ITS1/4 (White et al. 1990), and EF1- 728F/986R (Carbone and Kohn 1999). The amplified DNA products were sequenced in both directions using PCR primers (Macrogen, Inc., Seoul, Korea). The sequences were edited by Lasergene software package (DNASTAR, Madison, WI, USA) and compared with the sequences previously deposited in GenBank. NCBI-BLASTn analysis of the resultant sequences showed a high identify (EF1- α , 99.3; ITS, 100%) with reference sequence of *N. novaehollandiae* WAC 12691 (EF1- α , EF585574; ITS, NR111260). Also, the sequences were aligned by the

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Fig. 1 Disease symptoms caused by *Neoscytalidium novaehollandiae* associated with stem canker and branch dieback on almond tree. (a) branch die-back symptoms on almond tree, (b) vascular discoloration of the wood tissue (c) necrotic lesion on almond stem inoculated with the pathogen

MEGA 7.0 software package and used to construct a phylogenetic tree with Neighbor-joining method based on 1000 bootstrap replicates. A sequence of *Botryosphaeria dothidea* was selected as an outgroup (Fig. 2). The pathogen obtained from almond trees was closely related to *N. novaehollandiae* isolates from

different hosts. The sequences obtained were deposited in the GenBank database with accession numbers (ITS: MN982949, EF1- α : MN990035). The isolate An-2 was deposited in Erciyes University Culture Collection (EUCC WDCM1202) with the accession number EUCC-2920 M.

Fig. 2 Phylogenetic tree obtained by the neighbour-joining method from the partial sequences of nrDNA ITS and EF1- α gene regions of *Neoscytalidium* spp. isolates. *Botryosphaeria dothidea* is included as outgroup. Bootstrap values are shown as percentages of 1000 replicates

Pathogenicity tests were carried out on five 2-year-old almond trees cv. Ferragnes with the isolate An-2. The outer bark at the inoculation site was surface sterilized with 70% ethanol and a 5-mm diameter wound was created in the stem with a cork borer up to bark depth. A 5-mm-diameter disc from a 10-day-old culture on PDA was then placed on each wound and immediately covered with Parafilm. Sterile PDA plugs were deposited on the wounds of control plants. Inoculated plants were maintained in a greenhouse with a temperature range of 25 ± 2 °C. After 1-month, all inoculated plants showed necrotic lesions with a mean length of 3.3 cm. No symptoms were observed in the control plants. The pathogen was reisolated from lesions of inoculated stems, thus fulfilling Koch's postulates.

Neoscytalidium spp. described by Crous et al. (2006) was an important pathogen group associated with dieback and cankers of woody plants worldwide. *Neoscytalidium novaehollandiae* has been reported from different hosts such as *Mangifera indica*, *Adansonia gibbosa*, *Acacia synchronica*, *Crotalaria medicaginea*, and *Grevillia agrifolia* (Pavlic et al. 2008; Ray et al. 2010). The pathogen has been recently observed to cause wood canker of grapevine in Turkey (Akgül et al. 2019). However, to our knowledge, this is the first report of *N. novaehollandiae* associated with stem canker and branch dieback of almond trees.

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