First Report of *Colletotrichum nymphaeae* Causing Anthracnose on Almond in Hungary

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Almond (Prunus dulcis [Mill.] D. A. Webb) is cultivated in commercial orchards in southwestern Hungary while numerous backyard orchards predominate in Buda Hills and central Hungary. In July 2019, anthracnose symptoms and necrotic twigs were observed across almond genotypes in a meadow orchard of Óbuda and in the genebank collection of the Hungarian University of Agriculture and Life Sciences. Fruits of some genotypes were damaged 100%, whereas others to a lesser degree or asymptomatic. Orange slightly sunken lesions on fruits produced gum. Near the diseased fruits the young shoots shriveled, the stalks became necrotized, on twigs necrosis developed. Isolates obtained from orange conidial masses from epicarp, necrotized tissues from twigs, and stalks were grown on PDA for 7 days at 25 ˙C in the dark. Upper surfaces of the colonies were white to pale gray, black solid mycelial structures were formed, the reverse side varied white to salmon. Acervuli were not formed, but conidia were produced from hyphae. Conidia were unicellular, hyaline, smooth-walled, cylindrical, predominant with both end rounded, or one end acute. Morphometric measurements of conidia showed mean length ± SD × width ± SD = 18.0 ± 2.2 × 4.7 ± 0.6 µm (n = 100). The isolates were morphologically identified as *Colletotrichum acutatum sensu lato* (Damm et al. 2012). Sexual morph was not observed. Three monosporic isolates were used for molecular identification. Partial nucleotide sequences were amplified from three loci, internal transcribed spacer (ITS), β-tubulin (TUB2) and calmodulin (CAL) after White et al. (1990); Glass and Donaldson (1995) and Weir et al. (2012), respectively. The ITS sequences (GenBank accessions MW425388 to MW425390) of the three isolates revealed that they belong to the *C. acutatum* species complex while BLAST results showed that TUB2 sequences (GenBank accessions MW428285 to MW428287) had 99.3% identity with *C. nymphaeae* strain CBS515.78, whereas the CAL sequences (GenBank accessions MW428288 to MW428290) had 100% with *C. nymphaeae* strain FREC138. The phylogenetic tree containing all the valid species of *C. acutatum* species complex confirmed that the isolates clustered to *C. nymphaeae* with high bootstrap support. The fungus was identified as *Colletotrichum nymphaeae* (Pass) Aa based on molecular biological evidence. *In vivo* pathogenicity tests were conducted on ten healthy fruits, and ten twigs by inserting mycelial agar plugs (5 mm in diameter) onto wounded pericarp and phloem tissues, which were then wrapped in wet cotton and Parafilm®. The control treatments received sterile PDA discs. After 15 days, necrotic lesions 12 to 19 mm in diameter developed on fruit, 9 to 18 mm on twig. Control fruits, and twigs were asymptomatic. Koch's postulates were fulfilled with the reisolation of the pathogen from symptomatic tissues. The ITS, ACT and CAL sequences of the reisolated *Colletotrichum* were determined and found identical to the original isolates. Anthracnose symptoms are known on almond fruits in several almond growing regions all over the word caused by *Colletotrichum acutatum*, *C. godetiae*, *C. fioriniae*, *C. simmondsii*, and *C. gloeosporioides* (Adaskaveg et al. 1997; López-Moral et al. 2000; de Silva et al. 2021; Shabi et al. 1983;). To our knowledge, this is the first report of *Colletotrichum nymphaeae* causing anthracnose of almond globally.

References

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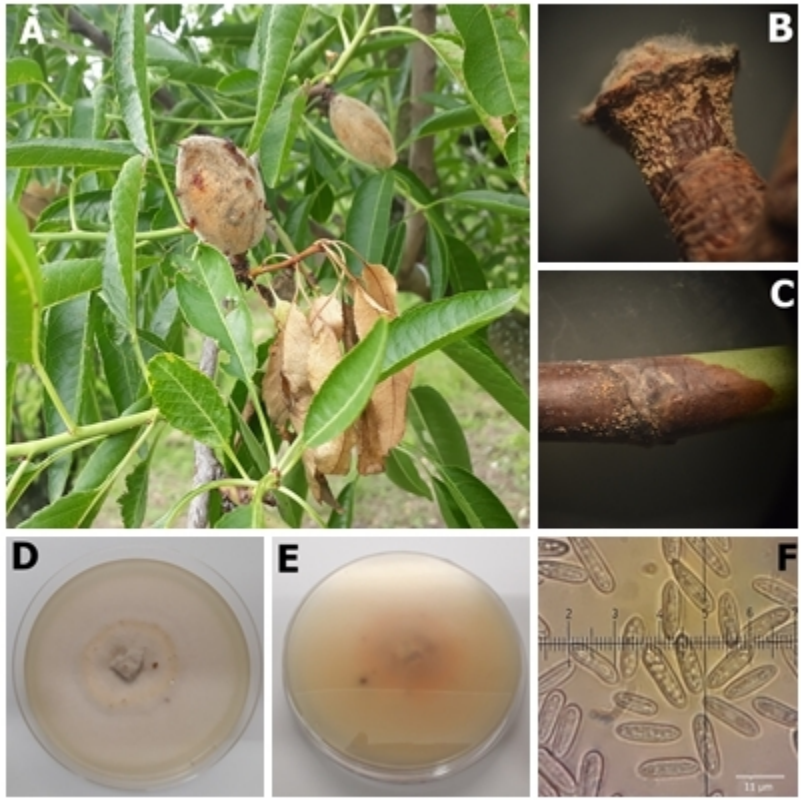
A: Symptom caused by Colletotrichum nymphaeae on almond fruit and shoots. B: Orange mass of conidia produced by acerculi on stalk of almond. C: Almond shoot colonized by Colletotrichum nymphaeae. D: Upper side of the colony. E: Reverse side of the colony. F: Conidia of Colletotrichum nymphaeae.

106x106mm (96 x 96 DPI)
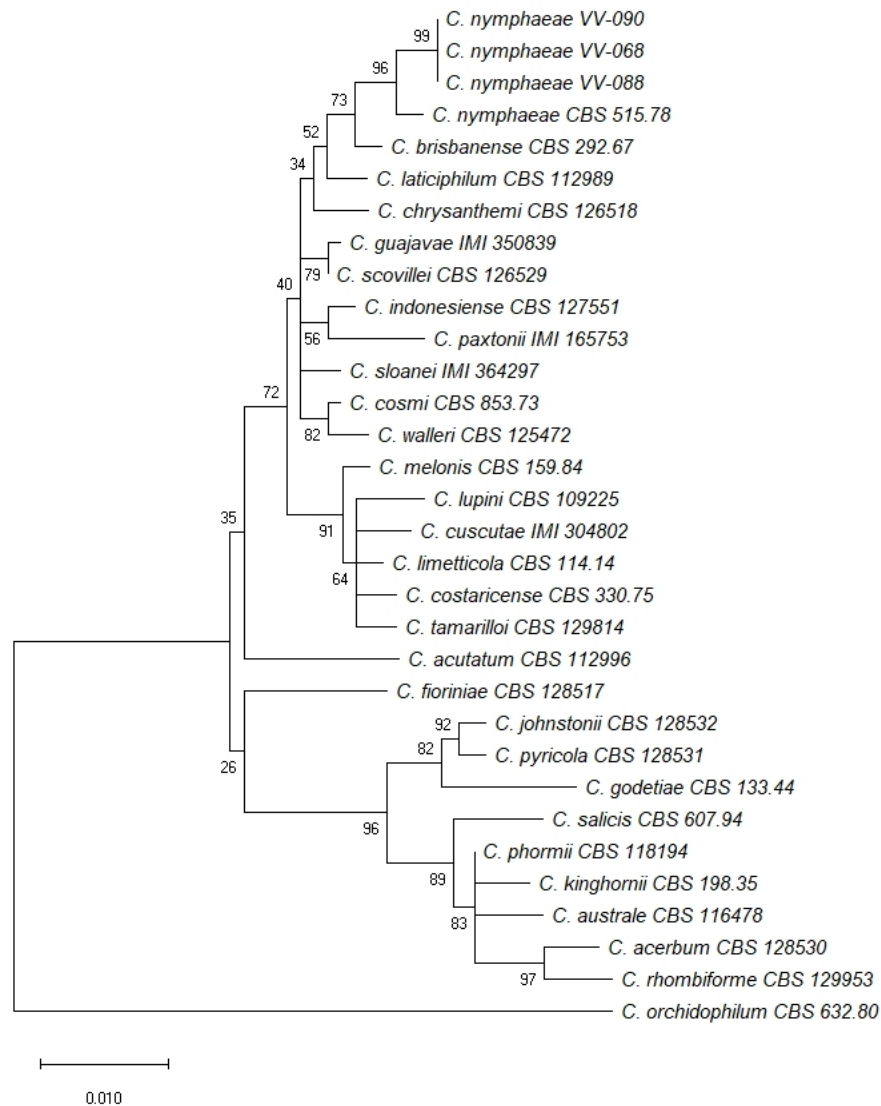
Evolutionary analysis of newly isolated 'almond' strains and all of the type strains of Colletotrichum acutatum species complex by Maximum Likelihood method. The phylogenetic tree was constructed using concatenated ITS and TUB2 sequences. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [1]. The tree with the highest log likelihood (-2661.09) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 32 nucleotide sequences. There were a total of 926 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2]. 1. Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526. 2. Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

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