

Citrobacter Braakii, a New Plant Pathogen, Causal Agent of Walnut Decline

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Abstract : BACKGROUND AND OBJECTIVES Walnut canker is characterized by brown to blackish roundish blotches on the trunks and main branches, necrosis of inner bark and bleeding with dark brown to black-colored exudates. The present study aimed to identify the causative agents of walnut decline by their phenotypic features, approval of pathogenicity, the partial sequencing of the housekeeping genes in Razavi Khorasan. MATERIAL AND METHODS Ten Symptomatic samples were collected from walnut orchards of Razavi Khorasan in 2019. Pathogenicity of all isolated strains was carried out on walnut immature fruits cv. 'Hartley' and young green twigs of cv. 'Chandler'. All pathogenic strains were subjected to physiological, morphological and biochemical tests. 16S rRNA and housekeeping genes (*fusA*, *leuS*, and *pyrG*) were partially amplified and sequenced. RESULTS Eight strains were able to cause necrosis and a dark-colored region in the mesocarp of immature walnut fruits, and three representative strains caused necrosis on young inoculated twigs. Strains utilized starch, however, did not utilize esculin, Tween 20, Tween 80, and gelatin. The partial 16S rRNA gene sequence of strain KH7 indicated 99.63 % similarity to that of *Citrobacter braakii* ATCC5113T. The phylogenetic analyses based on the partial sequencing of three housekeeping genes, *fusA* (633 bp), *pyrG* (305), and *leuS* (640 bp), demonstrated that strains KH1, KH3, and KH7 belong to *C. braakii* species in a monophyletic clade with high bootstrap support. CONCLUSION To the best of our knowledge, this is the first report of *C. braakii* as a new plant pathogen which cause walnut decline. Identification of bacteria associated with walnut decline will eventually improve our understanding of the etiology of the disease and may result in improved management techniques for control.

Keywords : emerging pathogens, Iran, *juglans regia*, MLSA

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