Agrobacterium tumefaciens causing crown and stem gall disease of citrus propagation nursery in Iran

Aram Ganjeh¹ · Heshmatollah Rahimian¹ · Esmaeil Basavand²

Received: 3 April 2020 / Accepted: 9 October 2020 / Published online: 19 October 2020
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Keywords *Agrobacterium tumefaciens* · *Citrus sinensis* · Pathogenicity · Molecular diagnosis

In 2015, overgrowths on the budding sites of a few growing budlings of sweet orange cv. Navelina and Okitsu mandarin on Carrizo citrange (*Poncirus trifoliata* × *C. sinensis*) and on two seedlings of this rootstock species were encountered on a citrus propagation nursery in Sari county of Mazandaran province, Iran. From fresh galls, the predominant colonies similar to *Agrobacterium* on sucrose nutrient agar (SNA) and D1M media were isolated 2–3 days after incubation of plates at 25–28 °C (Basavand et al. 2020). All isolates (ten in total) were gram negative, aerobic, motile and catalase and oxidase positive. They grew on 2% NaCl and at 35 °C. Isolates hydrolyzed urea and Tween-80 but not gelatin or starch. None were capable of producing a fluorescent pigment on King's B medium nor were able to produce indole or reduce nitrate. In pathogenicity test, the carrot (*Daucus carota*) discs in sterile Petri dishes and 3-months old citrange seedlings in greenhouse were inoculated by a bacterial suspension *ca.* 1×10^6 CFU/ml and were incubated at 26 °C (10 isolates with type strain *A. tumefaciens* ICMP 5856 T; 10 seedlings per isolate). Following 4 weeks after inoculation, callus symptoms on carrot discs and developing galls, 2 mm in diameter on citrange seedlings were observed. A sterile distilled water was utilized as control and the control plants remained symptomless. To fulfill Koch's postulates, re-isolation of inoculated tests isolates from citrange seedlings and carrot discs resulted on sucrose nutrient agar (SNA) and re-isolates were identified using conventional bacteriological methods. The 224 bp fragments, typical for a fragment of the *virD* gene of

the tumorigenic isolates and type strain *A. tumefaciens* (ICMP 5856 T) was amplified in PCR using the primer pair *virD2A/virD2C* (Bini et al. 2008). Further, part of the 16S rRNA gene of representative isolate (AR2) was amplified and sequenced (Weisburg et al. 1991). Thus, the nucleotide sequence (accession No. MT279042) shared 100% identity to that of *A. tumefaciens* (strain cqsm-fl) deposited in GenBank by BlastN analysis. To our knowledge, this is the first record of incidence of crown and stem gall caused by *A. tumefaciens* on citrus in Iran.

Acknowledgments The present study was supported by the Sari Agricultural Sciences and Natural Resources University, Iran.

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✉ Esmaeil Basavand
e.basavand@vru.ac.ir

¹ Department of Plant Pathology, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

² Department of Plant Pathology, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran