Diseases Caused by Bacteria and Phytoplasmas

Occurrence of Crown Gall Disease on Japanese Spindle (*Euonymus japonicus* var. Green Rocket) Caused by *Agrobacterium rosae* in Iran

Hamzeh Mafakheri,^{1,2} S. Mohsen Taghavi,¹ Sadegh Zarei,¹ Nemanja Kuzmanović,^{3,†} and Ebrahim Osdaghi^{2,†}

¹ Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz 71441-65186, Iran

² Department of Plant Protection, College of Agriculture, University of Tehran, Karaj 31587-77871, Iran

³ Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Plant Protection in Horticulture and Forests, Messeweg 11-12, 38104, Braunschweig, Germany

Funding: This study was supported by Shiraz University, Iran and Julius Kühn-Institut, Germany. Plant Dis. 106:313, 2022; published online as <https://doi.org/10.1094/PDIS-03-21-0580-PDN>. Accepted for publication 27 July 2021.

Crown gall disease caused by diverse *Agrobacterium* species is one of the main biotic constraints in the ornamental plants industry in Iran (Mafakheri et al. 2017). In August 2019, Japanese spindle (*Euonymus japonicus* var. Green Rocket) plants showing crown gall symptoms were observed in a commercial greenhouse in Tehran, Iran. Infected plants were characterized by a visible overgrowth on their stems and crown. Bacterial isolation from the gall tissues was performed on nutrient agar (NA) and 1A media as described by Moore et al. (2001). The six resulting bacterial strains (A.E1 to A.E6) were evaluated using PCR primer pair F8360/F8361 amplifying a 453-bp DNA fragment in the *recA* gene and confirmed as *Agrobacterium* sp. (Shams et al. 2013). Pathogenicity of the strains was evaluated in two independent assays on Japanese spindle plantlets as well as 10- to 15-day-old tomato (*Solanum lycopersicum* cv. Sunseed 6189) and sunflower (*Helianthus annuus* cv. Armavirski) plants in greenhouse conditions using the needle prick method as described previously (Mafakheri et al. 2019). The reference strain *A. radiobacter* ICMP 5856 and sterile distilled water were used as positive and negative controls, respectively. Crown gall symptoms appeared 20 to 25 days post inoculation on the Japanese spindle plantlets as well as tomato and sunflower plants inoculated with the strains isolated in this study, while the negative control plants remained asymptomatic. Koch's postulates were accomplished

by reisolating on NA medium and PCR-based identification of the inoculated strains from the symptomatic plants. The representative strain A.E1 was subjected to multilocus sequence analysis (MLSA) using the sequences of four housekeeping genes (i.e., *atpD*, *gyrB*, *recA*, and *rpoB*) as described previously (Mafakheri et al. 2019). MLSA results revealed that the strain A.E1 is phylogenetically closely related to *A. rosae*. The sequences were deposited into GenBank under the accession numbers MT007962 to MT007965 for *atpD*, *gyrB*, *recA*, and *rpoB*, respectively. Further, the strain A.E1 was subjected to whole genome sequencing using the Illumina HiSeq X platform. DNA extraction was performed using NucleoSpin Microbial DNA kit (Macherey-Nagel, Düren, Germany), DNA libraries were obtained with Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, U.S.A.), and de novo sequence assembly was performed using SPAdes genome assembler. The resulting whole genome sequence was deposited into the GenBank database under the accession number JAFJZW0000000000. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values were calculated among all the type strains of *Agrobacterium* species/genomospecies using standard criteria as detailed previously (Chen et al. 2021; Osdaghi et al. 2020). The strain A.E1 had 97% ANI and 72% dDDH values with *A. rosae* strain NCCPB 1650, suggesting that the bacterial strains isolated from Japanese spindle in Iran belong to *A. rosae*. This is the first report of *A. rosae* causing crown gall disease on Japanese spindle in Iran. The new crown gall disease could negatively affect the ornamental shrub production industry in central Iran unless strict sanitary measures are taken into the account in the nurseries in these areas. Further nationwide surveys and samplings are warranted to elucidate the economic impact of the pathogen on ornamental plant industry in the country.

References:

- Chen, G., et al. 2021. Phytopathology 111:268.
- Mafakheri, H., et al. 2017. Eur. J. Plant Pathol. 147:721.
- Mafakheri, H., et al. 2019. Phytopathology 109:1859.
- Moore, L. W., et al. 2001. Page 17 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad et al., eds. APS Press, St. Paul, MN.
- Osdaghi, E., et al. 2020. Appl. Environ. Microbiol. 86:e02873-19.
- Shams, M., et al. 2013. Syst. Appl. Microbiol. 36:351.

The author(s) declare no conflict of interest.

e-Xtra

Keywords: agrobacteria, MLSA, *Rhizobiaceae*, tumorigenesis, ornamental plants

[†]Indicates the corresponding authors.
N. Kuzmanović; nemanja.kuzmanovic@julius-kuehn.de,
and E. Osdaghi; eosdaghi@ut.ac.ir