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DISEASE NOTE

Stem and crown gall disease caused by *Agrobacterium tumefaciens* on Golden Euonymus in Iran

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In April 2016, samples of the stem and crown gall of Golden Euonymus with small to over 12 cm in diameter galls were collected from nurseries in the Mazandaran province, Iran (in total 10 symptomatic plants from 4 nurseries). From young galls, single colonies similar to *Agrobacterium* were isolated using conventional bacteriological methods (Basavand et al. 2020). Pathogenicity of 10 isolates with type strain was confirmed by stab inoculation of 11 tomato seedlings per isolate with a bacterial suspension of *ca*.1×10^7 CFU ml^{-1} and formation of galls on its stems in the following four weeks after inoculation. To fulfill Koch's postulates, re-isolation resulted on PDA + $CaCO_3$ medium and re-isolates were identified using standard bacteriological methods. Isolates (fifteen in total) were Gram- negative, catalase and oxidase positive, motile and aerobic. All isolates grew on 2% NaCl and at 35 °C, produced levan and urease and hydrolyzed Tween 80. They were negative in tests for production of fluorescent pigment, lecithinase, nitrate reduction, indole production, and hydrolysis of gelatin and starch. All isolates were capable of producing 3′-ketolactose and a pellicle on ferric ammonium citrate. They used dulcitol, arabitol, fructose, sorbitol, cellobiose, glycerol, lactate, xylitol and D-tartrate as source of carbon for growth. None of the isolates utilized β-alanine, valine, erythritol, L- tartrate, tyrosine, citrate or malonate. In all the identification tests, the type strain of *A. tumefaciens* (ICMP 5856) isolated from *Malus* was used as positive control. Results of biochemical assays were supported using the specific pair primer virD2A/virD2C in MLSA (Bini et al. 2008), and the *VirD* gene 224 bp fragment was amplified in 15 representative isolates by PCR. Moreover, the nucleotide sequence of the partial 16S ribosomal RNA of one representative isolate (Y15) using primer pairs FD1/RD1 (Weisburg et al. 1991) was amplified and sequenced. The sequence (accession No. LC143377.1) was highly (99%) similar to its corresponding sequence of *A. tumefaciens* isolates existing in GenBank. This is the first report of occurrence of stem and crown gall caused by *A. tumefaciens* on Golden Euyonymus in Iran.

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