Disease Note

THE FIRST REPORT OF A 16SrXII-A PHYTOPLASMA ASSOCIATED WITH TOMATO BIG BUD DISEASE IN IRAN

M. Salehi[1] and S.A. Esmailzadeh Hosseini[2]

[1]Plant Protection Research Department, Fars Agricultural and Natural Resources Research and Education Center, AREEO, Shiraz, Iran
[2]Plant Protection Research Department, Yazd Agricultural and Natural Resources Research and Education Center, AREEO, Yazd, Iran

In 2013 surveys, up to 7.5% incidence of big bud disease was observed in tomato fields of Kazerun area (Fars province, Iran). The main disease symptoms were big bud, little leaf, flower virescence, phyllody, proliferation and sterility. To investigate the phytoplasma presence, total DNAs extracted from four symptomatic and four symptomless tomato plants were tested by nested PCR using P1/P7 primer pair followed by R16F2n/R16R2 primers (Lee *et al*., 1998). Amplicons of *ca*. 1.8 and 1.2 kb, respectively were amplified in samples of symptomatic plants but not of symptomless ones. Four P1/P7 amplicons from symptomatic tomato plants were cloned and sequenced. The obtained 16S rDNA sequences showed 100% sequence identity with each other and a representative of these sequences deposited in GenBank (Accession No. KX098490). BLAST search using full length 16S rRNA gene sequence revealed that Kazerun tomato big bud (KTBB) sequence showed 100% identity with a *'Candidatus* Phytoplasma solani' strain (AF248959), representative of 16SrXII-A subgroup. Computer-simulated restriction analysis using *i*PhyClassifier showed that the RFLP profile of KTBB 16S rDNA F2nR2 fragment was identical (similarity coefficient 1.00) to the reference pattern of 16SrXII-A (AF248959). Phylogenetic analysis revealed that KTBB phytoplasma clustered with16SrXII group phytoplasmas closest to 16SrXII-A subgroup reference strain (AF248959). To our knowledge this is the first report of a 16SrXII-A phytoplasma associated with TBB disease in Iran. 16SrXII-related phytoplasmas have been previously reported from grapevine (Salehi *et al*., 2014) and *Cannabis sativa* (Vali Sichani *et al*., 2011) in Iran.

Lee I.-M., Gundersen-Rindal D.E., Davis R.E., Bartoszyk I.M., 1998. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology* **48:** 1153-1169.

Salehi E., Taghavi S.M., Salehi M., Izadpanah K., 2014. Partial biological and molecular characterization of phytoplasmas associated with grapevine yellows in Fars and Lorestan provinces of Iran. *Iranian Journal of Plant Pathology* **50**: 55-64.

Vali Sichani F., Bahar M., Zirak L., 2011. Characterization of stolbur (16SrXII) group phytoplasmas associated with *Cannabis sativa* witches'-broom disease in Iran. *Plant Pathology Journal* **10**: 161-167.

Corresponding author: M. Salehi
Fax:+9807133206376
E-mail: salehi_abarkoohi@yahoo.com

Received August 16, 2016
Accepted August 31, 2016

Disease Note

FIRST REPORT OF *PECTOBACTERIUM CAROTOVORUM* subsp. *BRASILIENSE* CAUSING BLACKLEG AND SOFT ROT OF POTATO IN TURKEY

M. Ozturk and H.M. Aksoy

Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey

In the summer of 2015, potato plants exhibiting blackleg symptoms were surveyed in 10 commercial fields in Amasya province in the Black Sea Region of Turkey. Average disease incidence was approximately 11% in the surveyed area, but could reach up to 40%. Stem tissue of diseased plants was homogenised and extract was plated on Nutrient Agar (Himedia, India). After 24 h incubation at 28°C single colonies were purified on Luria Agar (Himedia, India) or Crystal Violet Pectate (CVP) medium (Hyman *et al*., 2001). A total of nine strains that were cavity forming on CVP, gram-negative, catalase positive and facultative anaerobic with pectinolytic ability, produced a 434 bp product with *pel* gene specific primers Y1/Y2 (Darrasse *et al*., 1994) designed for *Pectobacterium* spp. One of these strains (A4G1) produced a 322 bp fragment typical for *Pectobacterium carotovorum* subsp. *brasiliense* using the subspecies specific primers (Br1f/Lr1) in the PCR assay (Duarte *et al*., 2004). Blastn analysis with a 1402 bp partial 16S rDNA gene sequence of strain A4G1 (GenBank Accession No. KX548227) showed 99% similarity to the 16S rDNA of *P. carotovorum* subsp. *brasiliense* strain 1001 (JF926759) at the nucleotide level. Phylogenetic tree analysis based on the Maximum Likelihood method, using 16S rDNA sequences avaliable in GenBank, clustered the two strains together. Surface sterilized whole potato tubers (cv. Marabel) were stabbed with a sterile pipette tip and inoculated with a 20 µl suspension of 10^8 cfu ml^{-1} of an overnight culture grown at 28°C. Inoculation wounds were covered with mineral oil to maintain partly anaerobic conditions in a dew chamber at 26°C. Strain A4G1 caused soft rot of tubers after 48 h incubation. Reisolated colonies caused pitting on CVP and exhibited the same morphology as original cultures on the NA plates. To our knowledge, this is the first report on the presence of *P. carotovorum* subsp. *brasiliense* in Turkey.

We are thankful to Prof. Dr. Ewa Lojkowska and Dr. Marta Potrykus for their kind helps during the interpretation of test results and important comments. This work was supported by project grant from OMU-PYO. ZRT. 1901.15.011. Thanks to TUBITAK BIDEB 2211-D Domestic Doctoral Fellowship Program.

Darrasse A., Priou S., Kotoujanski A., Bertheau Y., 1994. PCR and restriction fragment length polymorphism of a *Pel* gene as a tool to identify *Erwinia carotovora* in relation to potato disease. *Applied and Environmental Microbiology* **60**: 1437-1443.

Duarte V., De Boer S.H., Ward L.J., De Oliveira A.M.R., 2004. Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. *Journal of Applied Microbiology* **96**: 535-545.

Hyman L.J., Sulivan L., Toth I.K., Pérombelon M.C.M., 2001. Modified crystal violet pectate medium (CVP) based on a new polypectate source (Slendid) for the detection and isolation of soft rot erwinias. *Potato Research* **44:** 265-270.

Corresponding author: M. Ozturk
E-mail: muratzm66@gmail.com

Received August 29, 2016
Accepted September 20, 2016