**Disease Note**

# FIRST REPORT OF *LITTLE CHERRY VIRUS 2* ON *PRUNUS CERASUS* var. *MARASCA* IN CROATIA

**D. Vončina[1], S. Šimon[2], J. Ražov[3] and L. Leong[4]**

*[1]Department of Plant Pathology, University of Zagreb, Faculty of Agricultur, Zagreb, Croatia*
*[2]Syngenta Agro Ltd., Zagreb, Croatia*
*[3]Department of Plant Breeding Genetics, Biometrics and Experimentation, University of Zagreb, Faculty of Agriculture,Zagreb, Croatia*
*[4]Foundation Plant Services, Division of Agriculture and Natural Resources, University of California, Davis, USA*

*Little cherry virus 2* (LChV-2) is considered an important pathogen of cherries and, as such, testing of plant material is required according to the EPPO certification scheme PM 4/29 for cherries. In Croatia sour cherry Marasca is a well-known native variety with total production of 1500-1800 tons of fresh fruit per year, used mainly for production of renowned liqueurs and juices. In June 2014, 19 trees were selected and total RNA was extracted from leaves with the RNeasy plant mini kit (Qiagen, Germany). RT-PCR for LChV-2 was performed using two sets of primers: LCHV-2LO2/LCHV2UP2 (Rott and Jelkmann, 2001), amplifying a 438 bp fragment of the methyltransferase (MT) gene, and LC26L/LC26R (Eastwell and Bernardy, 2001), amplifying a 409 bp fragment of the RdRp gene. Six trees were positive, four with the first primer set and two with the second. However, none of the trees tested positive by both sets, suggesting the presence of significant sequence variability among LChV-2 isolates (Theilmann *et al*., 2004) and the presence of at least two virus variants in Croatia. One PCR product for each primer set was sequenced from both directions and sequences were compiled using MEGA6 software. BLAST searches indicated that the part of the MT gene of Croatian isolate M-79 (GenBank accession No. KT369315) shares 88% identity with the USA6b isolate of LChV-2 (AF531505), while isolate M-75 (KT369316) was closest to Canadian strain LC5 (AF416335), sharing 96% identity with part of the RdRp gene. During the 2015 harvest period, some of the LChV-2-infected trees displayed the characteristic symptoms of uneven ripening. To the best of our knowledge this is the first report of LChV-2 in Croatia.

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*Corresponding author*: D. Vončina
E-mail: dvoncina@agr.hr

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**Disease Note**

# FIRST MOLECULAR IDENTIFICATION OF '*CANDIDATUS* PHYTOPLASMA TRIFOLII' (16SrVI-D) IN *CROTON BONPLANDIANUM* FROM INDIA

**M. Priya[1], A.K. Tiwari[2] and G.P. Rao[1]**

*[1]Division of Plant Pathology, Indian Agriculture Research Institute, Pusa Campus-110012, New Delhi, India*
*[2]Central Lab, UP Council of Sugarcane Research, Shahjahnapur-242001, UP, India*

*Croton bonplandianum* Baill., a member of the family Euphorbiaceae, is a problematic weed throughout India. During 2013, phytoplasma like symptoms of leaf yellows, little leaf and witches' broom were observed in *C. bonplandianum* plants in the fields of the Sugarcane Research Station, Gorakhpur district of Uttar Pradesh (India). Leaves were collected from four symptomatic and three symptomless plants and DNA was extracted. Fragments of *ca.* 1.5 kbp of 16S rDNA were amplified from all four symptomatic samples in PCR assays using the phytoplasma specific primer pair P1/P6 (Deng and Hiruki, 1991) and the amplified product was sequenced (GenBank accession No. KJ410527). No amplification was achieved from any of the symptomless leaf samples. A pairwise 16S rDNA sequence comparison revealed the highest identity (99%) of *C. bonplandianum* phytoplasma strain with a member (JX104336) of the 16SrVI group ('*Candidatus* Phytoplasma trifolii'). The phylogenetic analysis of the 16S rDNA sequence of the Croton phytoplasma strain also revealed its close relationship with strains of '*Ca.* Phytoplasma trifolii'. The *ca.* 1.5k bp product of 16S rRNA sequence of *C. bonplandianum* phytoplasma strain submitted to *i*PhyClassifier online tool assigned it to the 16SrVI-D subgroup. Earlier, Tiwari *et al.* (2014) had reported the natural occurrence of '*Ca.* P. asteris' strain in ornamental croton (*Codiaeum variegatum*) in India, whereas Naik *et al.* (2015) reported a phytoplasma association with yellows and little leaf disease of *C. bonplandianum* in India on the basis of nested PCR amplifications but no group assignment was made. Hence, the present identification of 16SrVI-D subgroup phytoplasma associated with *C. bonplandianum* constitutes the first report worldwide.

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*Corresponding author*: Govind P. Rao
Email: gprao_gor@rediffmail.com

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