First report of \textit{Strawberry crinkle virus} in Argentina

M.C. Perotto\textsuperscript{1,2}, C. Luciani\textsuperscript{1,3}, M.G. Celli\textsuperscript{1}, A. Torrico\textsuperscript{1} and V.C. Conci\textsuperscript{1,2*}

\textsuperscript{1} Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias del Instituto Nacional de Tecnología Agropecuaria CP5119 Córdoba, Argentina; \textsuperscript{2} Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina; \textsuperscript{3} Fondo para la Investigación Científica y Tecnológica (FONCyT), Argentina

\textsuperscript{*}E-mail: conci.vilma@inta.gob.ar

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\textit{Strawberry crinkle virus} (SCV) is one of the most frequent viruses affecting strawberry worldwide, and responsible for important reductions in yield and fruit quality. Stunted dwarfed plants with distorted leaves were found in Lules (26°55′22″S 65°20′15″W), Tucumán province, Argentina, in 2010, suggesting the virus presence. Total nucleic acids were isolated from leaves of 26 strawberry plants (\textit{Fragaria x ananassa} cv. Camarosa) showing symptoms using the modified cetyltrimethylammonium bromide method (CTAB) as performed by Chang \textit{et al.} (2007). Healthy strawberry plants previously tested by grafting to indicator plants (\textit{Fragaria virginiana} clone UC-12, \textit{F. vesca} clone UC-6 and cv. Alpine) were used as negative controls.

The extracts were analysed by one-step nested RT-PCR (RT-PCR Kit, Qiagen) with specific primers (Posthuma \textit{et al.}, 2002), which amplify a 573-bp genome fragment corresponding to a conserved region of the rhabdovirus polymerase (L) genes. Two PCR products were cloned into the pCR4-TOPO\textsuperscript{®} vector (TOPO® TA Cloning Kit for Sequencing of Invitrogen Lab) and bi-directionally sequenced. In addition, new primers were designed based on the previously obtained sequences and others retrieved from the GenBank: Cito1/for: TCTATCAACCCTATGCAATATCCG; Cito1/rev: GTAGATATCTTCCAGCCACCTGATG (expected fragment size 744 nt) and Cito2/for: ATGGGACCTATGTACCGGACATC; Cito2/rev: GGAAATTGTGTCTCTCCCCATTG (687 nt) (Fig. 1). The PCR products obtained from each sample were cloned and bi-directionally sequenced. Sequences were assembled using the SeqMan program (Lasergene software, DNAStar ver. 5, 2001), and manual adjustment was done when necessary. The assembly of the three genomic fragments retrieved a consensus sequence (1897 nt), which was deposited in GenBank (Accession No. KJ748457). BLAST analysis showed that the Argentinian isolate has 95% (AY250986.2 - cover 100%) to 89% (JN542482.1 - cover 15%) nucleotide identity with SCV sequences (15). Phylogenetic analysis performed with a 258 nt fragment of 14 SCV sequences downloaded from the GenBank generated a tree in which the Argentinian isolate was grouped in group II according to Klerks \textit{et al.} (2004) (Fig. 2). Collectively, the data implicate the SCV as the etiological agent. To our knowledge, this is the first report of \textit{Strawberry crinkle virus} infecting strawberry in Argentina. Additional studies to complete the characterisation are being carried out.

References


Figure 1


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