Complete genome sequences of a Colombian and a Peruvian strain of Andean potato latent virus (APLV, genus Tymovirus) show that they are distinct virus species

De Souza, João A.; Cuellar, Wilmer J.; Kreuze, Jan F.

International Potato Center (CIP), Av. La Molina 1895 Lima – Perú.
International Center for Tropical Agriculture (CIAT), Apartado Aéreo 6713, Cali, Colombia.

Introduction

Andean potato latent virus (APLV) is a member of the genus Tymovirus in the family Tymoviridae, order Tymovirales (Gibbs et al., 1966; Fribourg et al., 1977). Despite its name, APLV can induce symptoms in infected potato plants, which however vary considerably depending on cultivars and growing conditions (Fribourg et al., 1977; Jones & Fribourg, 1978). Severe symptoms can also be induced in mixed infections with other potato viruses (Jones & Fribourg, 1978).

APLV was first described by Gibbs et al. (1966), as a virus consisting of isometric particles with a diameter of around 28 nm found in Colombia, Bolivia and Peru. The same isolate (denominated APLV-Col) was later found to be serologically related to Eggplant mosaic virus (EMV, Tymovirus; Gibbs & Harrison, 1969). Fribourg et al. (1977) characterized three further isolates originating from the Peruvian Andes: from Huancayo, Cajamarca y Ayacucho designating them as strains APLV-Hu, -Caj, -Ay, respectively and their presence was confirmed in Colombia, Peru, Bolivia and Ecuador. Furthermore, strain -Caj showed serological and thermal stability differences with -Hu and -Ay strains which were identical in that respect. Koening et al. (1979) categorized APLV isolates into three distinct serological groups according to gel diffusion assays (Koening et al., 1979). The isolates Col and Caj belonged to one group (Col-Caj), which is distributed throughout the Andes from Colombia to Ecuador, whereas the group CCC (Central Colombian potato Collection) was found only in accessions from the northern region of the Andes (Colombia), and the Hu group was found only in accessions from the central Andes (Southern Peru and Bolivia).

Objectives

Determinate the relationship between two isolates of APLV belong to different serological groups.

Materials and methods

For this work, two isolates of APLV were used, APLV-Hu conserved in the International Potato Center (CIP) and colombian isolate of APLV (APLV-Col) provided by Eng. César Fribourg. Total RNA was extracted from 1g of leaves of each plant using Trizol (Invitrogen), 50 ug of total RNA was run in 4% agarose gel with RNA marker (Biolabs), the bands located between 21 and 30 bp were excised and purified using the Gel Extraction Spin Columns Kit (Bio-Rad) and the obtained pellet was washed with 75% ethanol and dried at room temperature according to the procedure described by Kreuze et al. (2009). All siRNA were sent to Fasteris Life Science (Switzerland) for processing and sequencing on the Illumina Genome Analyzer. siRNA sequence reads were assembled using the AssemblyAssembler (v1.4) script included in Velvet (Zerbino and Birney, 2008) as described previously (Fuentes et al., 2012). Contigs were identified by BLAST against the Genbank and those corresponding to tymoviruses were extracted. Any gaps in the de-novo siRNA assemblies were separately sequenced by designing flanking primers, amplifying the corresponding regions and Sanger sequencing at Macrogen (Korea). Finally assembled complete sequences were re-confirmed by alignment of siRNAs using MAQ (Li, 2008; http://maq.sourceforge.net/index.shtml) Sequences were edited and annotated using the Vector NTI (Invitrogen) and CLC main workbench (v6.6.2; CLCBio) software packages. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011).
Results and discussions
From the bulk sample of 17 plants including one infected with APLV-Hu a total of 11,286,650 reads between 21-24 nts was obtained. A single contig of 6204nt with homology to tymoviruses could be assembled from the siRNA library reads using Velvet’s AssemblyAssembler and showed 99 % identity to the partial 3’ 1705 nt sequence of APLV available in Genbank (AF035402). From the bulk of three samples, one of which corresponded to siRNA from a plant infected with APLV-Col, a total of 1,305,287 reads between 21 and 24 nts were obtained. Eleven contigs ranging 1502-82 nt in length with similarity to tymoviruses were identified. Upon further analysis most contigs were found to overlap one of the others by 7-12 nts enabling us to create two super-contigs of 5643 nt and 686nt corresponding the 5’ and 3’ terminal parts of the virus. Primers were designed to adjoin the two sequences and the amplified product was sequenced and found to span a gap of 7 nts, between the two super-contigs.

When aligned to each other the two viruses showed only 60.59% nt identity, which is well below the species demarcation limit for Tymoviruses (80%; King et al., 2012). Indeed APLV-Col showed a similar level of nt identity to EMV (60.24%) and APLV-Hu was more similar to EMV (65.24%) than to APLV-Col. Phylogenetic analysis confirmed the results of sequence similarity showing that APLV-Hu and APLV-Col were clearly distinct viruses with APLV-Hu being more closely related to EMV. This report shows that viruses may have very similar or apparently even identical host ranges, but nevertheless differ considerably in their molecular and serological properties, which is consistent with Koenig et al. (2005). We suggest that APLV-Hu, an isolate belonging to the Hu group, be renamed South Andean potato latent virus (SAPLV), referring to its distribution which is restricted to the southern-Andes.

Conclusions
The complete genomes of APLV-Hu and APLV-Col isolates, which were sequenced in silico using siRNA obtained by deep sequencing, demonstrate that they are different viruses.

Acknowledgments
We are grateful to Eng. Agr. Mg.Sc. C. E. Fribourg for kindly supplying APLV-Col isolate. We thank to Hugo Espinoza for taking care of the plants. Financial support by QBOL and ICGEB-TWAS-UNESCO is duly acknowledged.

References