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Molecular detection and identification of group 16SrV and 16SrXII phytoplasmas associated with potatoes in Colombia

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Abstract

A severe disease was observed in the potato variety 'Criolla Colombiana'. Main symptomatology consisted of discoloration or yellowing of the whole plant, apical leafroll, dwarfing, axillary buds and thicker internodes. Phytoplasmas related to 16SrV and 16SrXII groups were identified by nested PCR assays followed by real and virtual RFLP and sequence analyses. This is the first report of phytoplasma presence in potato in Colombia and the first identification of group 16SrV phytoplasmas in this crop.

Key words: Potato, Colombia, phytoplasma, PCR/RFLP analyses, sequencing.

Introduction

Potato (*Solanum tuberosum* L.) is a staple food and one of the most important agricultural crops in Colombia, reaching about 162,000 ha with a production of more than 2,721.396 tons/year. Phytoplasma diseases of potato have been reported in several countries around the world where at least nine phytoplasmas were identified such as 16SrI-A, 16SrI-B, 16SrII, 16SrIII-M, 16SrVI-A, 16SrXII-A, 16SrXIII, 16SrXVIII-A and 16SrXVIII-B (Paltrinieri end Bertaccini, 2007; Santos-Cervantes *et al.*, 2010). In 2011 in Colombia phytoplasma-related sympotoms were found in potato for seed lots, multiplication in three areas in the municipalities of Guasca and Zipaquirá, (2,900 – 3,000 meters above sea level), in the variety 'Criolla Colombiana'. Symptoms of the disease are discoloration or yellowing of leaflets, apical leafroll, dwarfing, axillary buds and thicker internodes (personal communication FEDEPAPA, 2011). Molecular analyses were carried out to verify phytoplasma association with this disease.

Materials and methods

Symptomatic leaves and stem of potatoes were collected from eight naturally diseased plants exhibiting symptoms, in the location of Guasca and Zipaquirá in the state of Boyacá. Total nucleic acids were extracted from 1 g of mixture of leaf midribs and stem phloem tissues of eight samples, dissolved in Tris-EDTA pH 8 buffer, and maintained at 4°C; 20 ng/µl of nucleic acid were used for amplification. Universal and specific primer pairs P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) and R16F2/R2 (Lee *et al.*, 1995), and R16(I)F1/R1, R16(V)F1/R1(Lee *et al.*, 1994) were used to prime amplification of phytoplasma 16S rDNA sequences in nested and second nested PCR assays.

Table 1. Results of PCR/RFLP analyses on potato.

Plant	Tissue	PCR/RFLP		
		$16R_{758f}$ /$16R_{1232r}$	R16(I) F1/R1	R16(V) F1/R1
1	Leaf	16SrV+ -XII	16Sr	16SrV
	Stem	-	-	16SrV
2	Leaf	16SrXII	-	16SrV
	Stem	16SrXII	-	16SrV
3	Leaf	16SrV	16SrXII	16SrV
	Stem	16SrXII	16SrXII	16SrV
4	Leaf	16SrXII	16SrXII	16SrV
	Stem	-	-	16SrV
5	Leaf	-	-	16SrV
	Stem	-	-	16SrV
6	Leaf	-	-	16SrV
	Stem	16SrV	-	16SrV
7	Leaf	16SrXII	-	16SrV
	Stem	16SrV	-	16SrV
8	Leaf	16SrV	-	16SrV

-, no phytoplasma detected

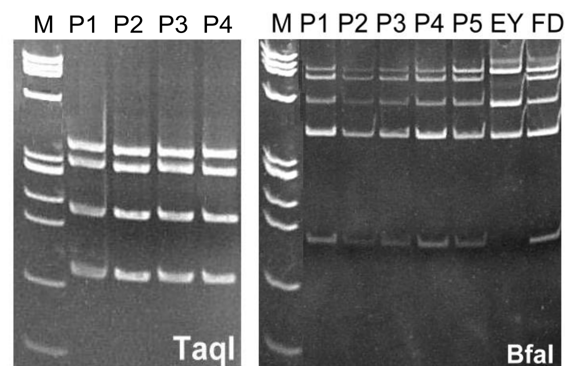

Figure 1. RFLP analyses of R16(V)F1/R1 and $16R_{758f}$/V1731 amplicons. P, potatoes samples, EY, elm yellows (16SrV-A); FD, 'flavescence dorée' (16SrV-C). M, marker ΦX174 *Hae*III digested.

Further nested PCR were performed with $16R_{758f}/16R_{1232r}$ (Gibb *et al.*, 1995) and $16R_{758f}$/V1731 (Martini *et al.*, 1999) primer pairs. All PCR reactions were carried out following the protocol of Schaff *et al.* (1992). Phytoplasma identification was done with RFLP analyses with *Tru*I, *Bfa*I, and *Taq*I, (Fermentas, Vilnius, Lithuania) restriction enzymes according with amplicons employed. Selected R16(V)F1/R1 and $16R_{758f}$/V1731 amplicons were purified using Nucleospin extract (Macherey-Nagel, Germany) and directly sequenced in both directions using the BIG DYE sequencing terminator kit (PE Biosystems, Warrington, UK). The sequences were then aligned with BLAST engine for local alignment (http://www.ncbi.nim.nih.gov/ BLAST/). 16S rDNA sequences were retrieved from NCBI and used to construct phylogenetic trees. Minimum evolution analysis was carried out using the neighbor joining method and bootstrap replicated 500 times with the software MEGA 4.1 (http:// www.megasoftware.net /index.html) (Kumar *et al.*, 2004). *Acholeplasma palmae* was used as the outgroup. To rule out other pathogens possibly associated with the disease ELISA tests for PLRV, PVX, PVY and PVS viruses were carried out.

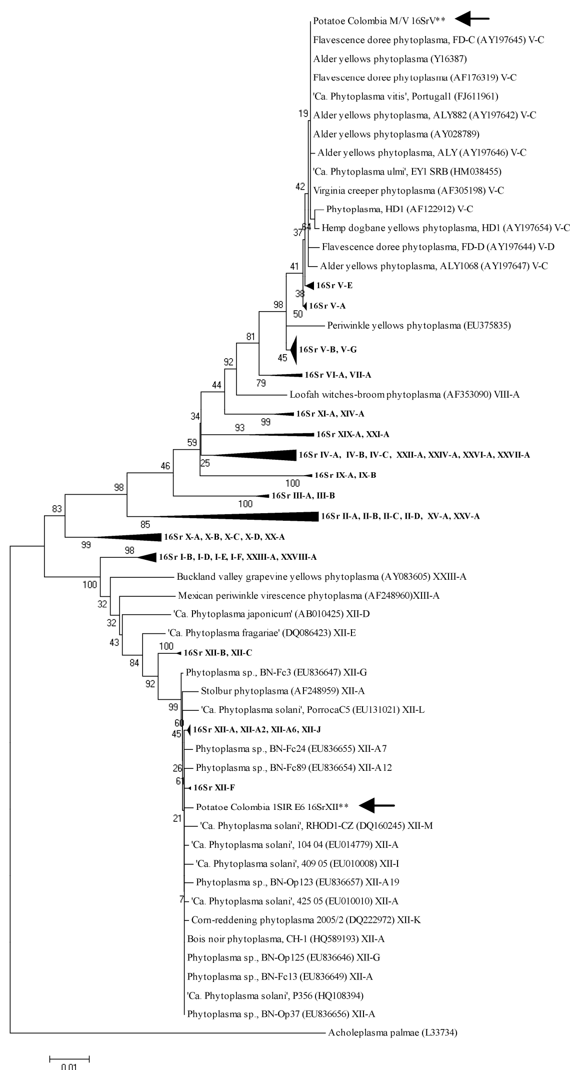

Figure 2. Phylogenetic tree from phytoplasma partial-length 16S rDNA. Sequences determined in this study are indicated by asterisks and arrows.

Results and discussion

All potato samples were negative in the tests for virus detection and positive for phytoplasma presence; RFLP analyses confirm the presence of 16SrXII phytoplasmas, while in samples amplified with 16SrV-group specific primers phytoplasmas related to 16SrV-C subgroup was determined by both RFLP analyses and sequencing (table 1; figure 1 and 2). In particular 16SrXII phytoplasmas were identified in 7 samples always in mixed infection with 16SrV phytoplasmas. This new potato disease reported in Colombia could cause severe economic losses since potato seed lots are grown in areas where high incidence of the disease has been already reported. This is the first identification of 16SrV phytoplasmas in potato and of its mixed infection with stolbur in Colombia and worldwide.

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