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Study of stolbur phytoplasma tuber transmission in potato varieties of high starch content

Ibolya EMBER, Cecilia TALABER, Zoltan ACS, Zita NAGY, Maria KÖLBER
Fitolab Plant Pest Diagnostic and Advisory Ltd., Budapest, Hungary

Abstract

In order to study the tuber transmission rate of stolbur phytoplasma tubers from varieties of high starch content, originating from field under high infection pressure, and their daughter plants were tested with PCR-RFLP and real-time PCR. Altogether 83.8% of the 702 tubers tested were positive for stolbur. During the three-years surveys the phytoplasma was identified in 0.5% of daughter plants. These data confirm that tuber transmission of stolbur phytoplasma also in varieties with high starch content although in a low percentage. Further studies are needed to discover factors influencing the tuber transmission ability of the stolbur phytoplasma.

Key words: potato stolbur, epidemiology, purple top, molecular detection.

Introduction

Potato stolbur phytoplasma is widespread in central and southern part of Europe causing yellows-type diseases on solanaceaous crops (Cousin and Smith, 1977; EPPO/CABI, 1996). At least five phytoplasmas belonging to three different groups have been associated with potato diseases. Stolbur, potato purple top (PPT) and potato witches' broom (PWB) are the most important diseases. Stolbur disease is associated with phytoplasma belonging to 16SrXII group. Transmission of different phytoplasmas via potato tubers has been reported by only a few authors so far. Tuber transmission was proved by Paltrinieri and Bertaccini (2007) in case of stolbur phytoplasma as well as of phytoplasmas belonging to ribosomal subgroups 16SrI-B, 16SrI-C, 16SrII-D and 16SrX-A. In the USA tuber transmission was reported by Munyaneza and Crosslin (2006) in 35% of the plants grown out from tubers infected by beet leafhopper transmitted virescence (BLTVA) phytoplasma (16SrVI-A), associated with the PPT disease.

Potato varieties are categorized into groups based on common characteristics that are based on starch value. For processing varieties high starch content (17 to 20%) is required compared to the table once (10-14%) (Potato Council, 2009).

For better understanding the epidemiology of the potato stolbur, it is necessary to determine the tuber transmission rate in case of different varieties.

The objective of the present work was to verify the existence and rate of stolbur phytoplasma tuber transmission in high starch content potato varieties.

Materials and methods

Tubers of four varieties of high starch content (Lady Rosetta and varieties A, B and C) were collected from stolbur-infected field in Romania. Tubers were harvested after natural loss of foliage in August. Tubers were planted in a growing room in April 2009, in January 2010 and 2011. Visual observation of the plants was carried out regularly.

Samplings for molecular tests were performed at three times in 2009, 2010 and twice in 2011. The first test was made on the tubers (before planting out), the second test on daughter plants at four-week age and the third test at three-month age. DNA extraction from 702 tuber samples and 629 plant samples were carried out using the CTAB method (Daire *et al.*, 1997). For tubers, nested PCR was applied using primers P1/P7 (Deng and Hiruki, 1991; Smart *et al.*, 1996) and R16F2/R16R2 (Lee *et al.*, 1995) amplifying 16SrDNA. The nested products were digested with *Tru1*I enzyme and the RFLP profiles were compared with reference strains (EAY, 16SrI-B; MOL, 16SrXII-A provided by A. Bertaccini and AY27, 16SrI-A; CPh, 16SrI-C; PaWB, 16SrI-D; CP and PWB, 16SrVI-A from I-M. Lee).

Results and discussions

Symptomatology

Tubers. The average diameter of the tubers was 3-4 cm. Altogether 81.8% of the 702 tubers were found symptomatic, 30.9% showed hairy sprouts, 43.3% were spongy and 27.9% showed both symptoms; the percentage varied among the varieties (data not shown).

Daughter plants. The first typical phytoplasma symptoms appeared already at the four-week stage. Purple top, yellowing, stunting, uprolled crispy leaves and aerial tubers developed on one Lady Rosetta and two Variety A plants during the three-year experiment. These three plants were very small, stunted and did not develop daughter tubers (data not shown).

Molecular results

Tubers. Altogether 83.8% of the 702 tubers proved positive for stolbur (16SrXII) (table 1). Stolbur was identified in symptomatic and asymptomatic tubers as well. The severely shrunken, small tubers were negative for phytoplasma.

Table 1. Stolbur infection rate of tubers and daughter plants.

Variety	Infected tubers before planting infected/tested (%)			Infected daughter plants four-week old infected/tested (%)			Infected daughter plants three-month old infected/tested (%)	
	2009	2010	2011	2009	2010	2011	2009	2010
Lady Rosetta	86/118 (73.0)	74/110 (67.3)	92/100 (92.0)	0/104*	0/99	1/77 (1.3)	1/73* (1.4)	0/101
Variety A	/	48/54 (88.9)	90/100 (90.0)	/	1/44 (2.3)	1/80 (1.25)	/	1/52 (1.9)
Variety B	/	104/110 (94.5)	/	/	0/100	/	/	0/108
Variety C	/	94/110 (85.5)	/	/	0/66	/	/	0/107
3-year total	Lady Rosetta: 252/328 (76.8) Variety A: 138/154 (89.6) Variety B: 104/110 (94.5) Variety C: 94/110 (85.5)			Lady Rosetta: 1/280 (0.4) Variety A: 2/124 (1.6) Variety B: 0/100 (0.0) Variety C: 0/66 (0.0)			Lady Rosetta: 1/174 (0.5) Variety A: 1/52 (1.9) Variety B: 0/108 (0.0) Variety C: 0/107 (0.0)	

* = in 2009 the number of plants were decreasing due to plant death;
/ = not tested.

Daughter plants. Stolbur phytoplasma was identified only in symptomatic plants (table 1): in one of Lady Rosetta (Ct: 32.5) and in two of Variety A (Ct: 31.4, 34.2). Signal of endogenous control was measured in 99% of the tested samples (Ct values: 18.5-22.9) (data not shown).

The three-year average of infection rate, calculated on data of the four-week old daughter plants, was 0.4% for Lady Rosetta and 1.6% in case of Variety A.

The rate of tuber transmission, expressed in three-year average of the four potato varieties, was 0.5% (table 1).

These results confirm a low percentage of stolbur phytoplasma tuber transmission also in varieties with high starch content.

It can be assumed that the tuber transmission of stolbur phytoplasma may have a low epidemiological impact in the tested varieties. However the importance of stolbur tuber transmission may not be leaved out of consideration in case of other varieties. Further studies are needed to discover factors that may influence the tuber transmission ability of stolbur such as biological characters of the varieties as starch content, storage conditions or planting date.

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Corresponding author: Ibolya EMBER (e-mail: ember.ibolya@fitolab.hu) Fitolab Ltd., H-1125 Istenhegyi ut 29. Budapest, Hungary.