Bulletin of Insectology ***60*** *(2): 379-380, 2007*
ISSN 1721-8861

Detection of phytoplasmas in plantlets grown from different batches of seed-potatoes

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Abstract

During 2006 and 2007 eight batches of seed potatoes collected in different locations, and belonging to one cultivar were planted in spring under greenhouse conditions and tested after 2 months to verify phytoplasma presence. A total of 635 asymptomatic plantlets were examined. Nucleic acid was extracted from small shoots from either a single plant or from batches of 3 plants each. Nested PCR on both single and grouped samples with general ribosomal primers, without spacer region allowed specific phytoplasma detection. Phytoplasmas belonging to diverse ribosomal groups were identified after RFLP analyses according to the batch tested. Ribosomal subgroups 16SrI-B (related to '*Candidatus* phytoplasma asteris'), 16SrI-C (related to clover phyllody: CPh), 16SrII-D (related to tomato big bud from Australia: TBB), 16SrX-A (related to '*Ca*. P. mali'), and 16SrXII-A (related to stolbur) were identified in different percentages. After further validation tests, the system can be used to screen high quality seed potatos for phytoplasmas.

Key words: molecular detection, phytoplasmas, potatoes, epidemiology.

Introduction

Potato is susceptible to a large number of plant pathogens. Among them, bacteria, nematodes, viruses and viroids are the most studied, while phytoplasma-associated diseases have been less extensively examined. Several diseases are reported to be caused by phytoplasmas worldwide; the most important are potato purple top (PPT), potato witches' broom (PWB), and potato stolbur occurring respectively in the Americas and in Eastern Europe (Kadhair *et al*., 1997; Lee *et al*., 2006; Leyva-Lopez *et al*., 2002). At least five distinct phytoplasmas were identified. While phytoplasmas associated to PWB belong to ribosomal subgroup 16SrVI-A, PPT disease is associated with several phytoplasmas belonging to ribosomal groups 16SrI, 16rII, 16SrVI, and XVIII. Stolbur disease is associated with phytoplasmas belonging to subgroup 16SrXII-A (*sensu* Vibio *et al*., 1996; OEPP/EPPO, 2004). Since global movement of planting material is becoming more common, a survey was carried out in order to verify the sanitary status of seed potatoes used for plantations in Italy and to set molecular procedures for early detection of potato phytoplasmas. Prevention of the spread of pathogens not present in agricultural areas is the best tool to avoid dangerous outbreaks of phytoplasma diseases in potatoes as well as in other susceptible crops.

Materials and methods

During 2006 and 2007 eight batches of seed potatoes collected in different locations, and belonging to one cultivar were planted in spring under greenhouse conditions and tested after 2 months to verify phytoplasma presence. A total of 635 plantlets were examined, all were asymptomatic. Nucleic acid was extracted from small shoots (2-15 cm long) collected from either a single plant or from batches of 3 plants each. Two different extraction methods were employed: one was based on chloroform/phenol for 2006 samples (table 1) (Prince *et al*., 1993) and the other was using CTAB and chloroform for 2007 samples (table 2) (Angelini *et al*., 2001).

After preliminary tests using universal primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) in direct PCR followed by nested PCR with R16F2/R2 and R16(I)F1/R1 (Lee *et al*., 1995), R16mF2/mR2 (Gundersen and Lee, 1996) primers were employed in direct PCR. Cycles and conditions were as published (Schaff *et al*., 1992). RFLP analyses for phytoplasma identification were performed with *Tru*I and *Taq*I (Fermentas, Vilnius, Lithuania) for 16 hours under conditions described from the manufacturer. Polyacrylamide 5% gels stained with ethidium bromide were employed to compare profiles to reference phytoplasmas (Bertaccini *et al*., 2000).

Results

Only the use of nested PCR allowed the detection of phytoplasmas in both single and grouped samples from potato plantlets. The type of phytoplasma present varied between the different potato batches (tables 1 and 2). Phytoplasmas belonging to diverse ribosomal groups were identified after RFLP analyses on 16S rDNA gene. The tests classified the phytoplasmas as belonging to ribosomal subgroups 16SrI-B (related to '*Candidatus* Phytoplasma asteris'), 16SrI-C (related to Clover phyllody: CPh), 16SrII-D (related to tomato big bud from Australia: TBB), 16SrX-A (related to '*Ca*. P. mali'), and 16SrXII-A (related to stolbur). The use of primers R16mF2/mR2, that only amplify 16S rDNA from phytoplasmas, eliminates the aspecific amplification obtained in nested PCR with the R16F2/R2 or R16(I)F1/R1 when direct PCR was carried out with P1/P7primers. The presence of aspecific bands was revealed by RFLP profiles observed after digestion of bands of the expected size.

Table 1. Results of molecular tests to identify phytoplasma presence and identity in batches of 3 seed potato plantlets from different locations (2006). (*) estimated percentages for single shoot.

Batches	Number of samples	% of phytoplasmas (*)	% of identified phytoplasmas				
			16SrI-B (*)	16SrI-C	16SrII-D	16SrX-A	16SrXII-A (*)
A	37	2.70 (0.90)	-	-	-	-	2.70 (0.90)
B	34	26.50 (9.69)	2.94 (0.98)	-	-	-	23.53 (8.48)
C	32	28.13 (10.33)	6.25 (2.11)	-	-	-	21.88 (7.83)
D	34	5.88 (1.98)	-	-	-	-	5.88 (1.98)
E	33	9.00 (3.07)	9.00 (3.07)	-	-	-	-
F	16	62.50 (27.66)	12.50 (4.32)	-	-	-	50.00 (20.45)
TOTAL	186	17.74 (6.25)	4.30 (1.44)				13.44 (4.66)

Table 2. Results of molecular tests to identify phytoplasma presence and identity in batches of single seed potato plantlets from different locations (2007).

Batches	Number of samples	% of phytoplasmas	% of identified phytoplasmas				
			16SrI-B	16SrI-C	16SrII-D	16SrX-A	16SrXII-A
G	46	28.29	6.52	6.52	4.35		10.90
H	31	41.94			32.26	3.23	6.45
TOTAL	77	33.68	3.90	3.90	15.58	1.30	9.00

Discussion

The phytoplasma detection in asymptomatic potato plantlets grown under protected conditions indicates that these pathogens can be latently present in the propagating material. The presence of stolbur and tomato big bud related phytoplasmas is in agreement with literature reports (OEPP/EPPO, 2004; Lee *et al*., 2006) even if usually these phytoplasmas were until now only identified in severely symptomatic plants. The finding of phytoplasmas related to '*Ca*. P. mali' is new for this species and one of the few reports of this phytoplasma in a herbaceous host. Until now, it was only reported erratically in clover in the Czech Republic.

The protocol employed in this study for phytoplasma detection reflects the OEPP/EPPO (2004) protocol but introduces the reliable and sensitive use of a nested PCR system that could be applied, after further validation tests, to screen high quality seed potatoes for phytoplasmas.

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