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The use of groEL gene for characterisation of aster yellows phytoplasmas in field collected samples

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Abstract

Amplification of fragments containing phytoplasma groEL gene sequence with a newly designed nested PCR system allowed to specifically detect the presence of '*Candidatus* Phytoplasma asteris' in reference strains as well as in samples field collected and maintained as dry/freeze dried nucleic acids. After RFLP analyses it was possible to confirm further finer differentiation among strains enclosed in subgroup 16SrI-B by previous ribosomal gene classification.

Key words: phytoplasmas, PCR/RFLP analyses, groEL gene, molecular characterization, epidemiology.

Introduction

Phytoplasma classification established using 16S ribosomal groups and '*Candidatus* Phytoplasma' taxon is mainly based on 16S rDNA properties and do not always provide molecular distinction of the closely related strains such as those in the aster yellows group (16SrI or '*Candidatus* Phytoplasma asteris' - related strains). More variable single copy genes, such as ribosomal protein (rpl22 and rpS3), secY, tuf, and groEL were employed for finer aster yellows phytoplasma differentiation (Marcone *et al*., 2000; Lee *et al*., 2004; 2006; Martini *et al*., 2007; Mitrović *et al*., 2011). A nested PCR system was developed on groEL gene and it was tested on aster yellows phytoplasma strains from field infected samples from different plant hosts and different geographical locations toward epidemiological purposes.

Materials and methods

Nucleic acid samples extracted from 1992 to 2010 from 36 symptomatic plants of different species identified as 16SrI phytoplasmas by PCR/RFLP analyses (Lee *et al*., 1998), and maintained as dry or freeze dry pellet at 4°C or -20°C respectively, were employed. The 36 nucleic acid samples (table 1) were employed for amplification in direct PCR with newly designed primers AYgroesF (ATCAGAAAAAGAAAAATCCT) and AYampR (GCAACAGCAGCAAATAAAAC) that amplify a region of about 2,100 bp external to AYgroelF/AYgroelR (Mitrović *et al*., 2011). Nested PCR was then carried out on amplicons diluted 1: 30. Reference strains employed for preliminary specificity assays were aster yellows from France (AY-J, 16SrI-B), primrose virescence from Germany (PRIVA, 16SrI-B) stolbur from pepper from Serbia (STOL, 16SrXII-A), and tomato big bud from Australia (TBB, 16SrII-D) in periwinkle, and on '*Ca.* P. japonicum' in *Hydrangea* sp. (16SrXII), 'flavescence dorée' in grapevine from Serbia (FD, 16SrV-C), and European stone fruit yellows in peach from Serbia (ESFY, 16SrX-B). Each 25 µl PCR reaction mix contained 20 ng template DNA, 5 U of Sigma-*REDTaq* ® DNA *Polymerase* (Sigma-Aldrich Co.), 2.5 µl of its 10X buffer, 2 µl d-NTPs (Fermentas, Vilnius, Lithuania) and 0.4 µM of each primer. Samples lacking DNA were employed as negative controls. Thirty-five PCR cycles were performed for both primer pairs as previously described (Mitrović *et al*., 2011). The groELI (groEL gene RFLP group I) RFLP subgrouping was performed with *Alu*I and *Tru*I restriction enzymes (Fermentas, Vilnius, Lithuania). Restriction products were separated in 5% polyacrylamide gel and profiles were compared with those obtained from positive reference strains.

Results and discussion

Expected length amplicons (about 1.4 kb) of partial *groEL* gene were obtained only with reference strains belonging to aster yellows group (16SrI) confirming specificity of the nested PCR system employed to 16SrI phytoplasma group. All 36 field collected samples were amplified producing the expected length amplicons and RFLP analyses with *Tru*I and *Alu*I restriction enzymes yielded six and seven different profiles respectively according to Mitrović *et al.* (2011) (table 1) allowing differentiation of AY phytoplasmas to eight groELI RFLP subgroups, of which two were newly identified: one in cabbage and one in periwinkle. The developed method allows detection of aster yellows strains from field collected samples allowing discrimination that can be of epidemiological relevance such as in the cases of phytoplasmas in carrot, onion, oil palm and corn.

Table 1. Results from AYgroelF/AYgroelR nested PCR amplification and RFLP analyses for fine characterization of aster yellows phytoplasmas from field collected samples.

Phytoplasma disease	Geographical location	Number of tested samples (year)	RFLP literature	Grouping			
				*Tru*I	*Alu*I	groELI	16SrI
Papaver virescence	Forlì (Italy)	1 (2009)	This paper	1	1	III	-B
Oil seed rape phyllody	Padova (Italy)	6 (2009)	Mori *et al*., 2010	1	1	III	-B
Onion phyllody and virescence	Potenza (Italy)	4 (1994)	Vibio *et al*., 1995	1 2	1 2	III IV	-B -B
Cabbage phyllody	Ravenna (Italy)	6 (1994)	Bertaccini *et al*., 1998	1 7	1 1	XI	-B
Lettuce yellows	Florence (Italy)	1 (2000)	This paper	1	1	III	-B
Lettuce yellows	Imperia (Italy)	5 (1992)	Vibio *et al*., 1994	1	1	III	-B
Oil palm 'machite lethal'	Colombia	1 (2004)	-	2	8	V	-B
Corn stunt	Palmira (Colombia)	2 (2007)	Duduk *et al*., 2008	2	8	V	-B
Periwinkle virescence	Belgrade (Serbia)	2 (2010)	This paper	4	4	VI	-C
Periwinkle virescence	Torino (Italy)	1 (1998)	This paper	1	1	III	-B
Periwinkle virescence	Saint Louis, USA	1 (1994)	This paper	1	9	X	-B
Periwinkle virescence	Ferrara (Italy)	1 (1998)	This paper	1	1	III	-B
Carrot proliferation	Begeč (Serbia)	3 (2006)	Duduk *et al*., 2009	1 2 3	1 2 3	III IV I	-B ? -A
Populus decline	Belgrade (Serbia)	1 (2009)	Mitrović *et al*., 2011	6	7	IX	-P
Grindelia virescence	Ravenna (Italy)	1 (2008)	Bertaccini *et al*., 2011	2	2	IV	-B

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