Bulletin of Insectology ***60*** *(2): 307-308, 2007*
ISSN 1721-8861

Occurrence of '*Candidatus* Phytoplasma mali' in apple cultivars or selections grown in Trentino (North Italy) orchards

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Abstract

Severe apple proliferation (AP) epidemics are spread in the most important apple growing areas in Europe with a significant economic impact on the production of marketable fruits. A genotype screening was undertaken on 41 apple cultivars and selections showing no phytoplasma-related symptoms to identify natural resistance or tolerance to '*Candidatus* Phytoplasma mali'. Among the tested cultivars or selections most of asymptomatic trees of cultivars Brina and Enova resulted to be positive to phytoplasma presence, while in selection CO-OP12 only one out of 34 tested plants resulted positive to AP infection.

Key words: Apple proliferation phytoplasmas, screening for resistance, molecular detection.

Introduction

Apple proliferation (AP) is a phytoplasma associated disease firstly described in Italy in the fifties (Rui *et al.*, 1950, Refatti and Ciferri, 1954) but showing increasing epidemic activity in the last 15 years. This disease affects size, weight and quality of fruits, as well as tree vigour and it increases susceptibility to powdery mildew. Almost all main apple cultivars, *i.e.* 'Golden Delicious', 'Renetta Canada', 'Red Delicious', grown in Trentino valleys are susceptible and no curative treatments are currently available.

The phytoplasma associated with this disease has been recently classified as '*Candidatus* Phytoplasma mali' (Seemüller and Schneider, 2004). In order to contain the epidemic impact of AP in large apple growing areas, researches were undertaken to study the apple genetic background with respect to '*Ca.* P. mali' susceptibility. Towards this aim a screening was performed on cultivars or selections available in field collections maintained in areas in which the AP epidemic is present for more than 10 years. Genotypes were selected among those that did not show disease symptoms since several years of observations.

Materials and methods

The research was carried out in Trentino Alto Adige region (North Italy) and samples were collected in the apple germplasm collection maintained by C.R.A Fruit Trees Research Institute in Pergine Valsugana and in near apple growing areas. Asymptomatic trees of the following cultivars or selections were tested for the presence of '*Ca.* P. mali': Brina, CLR13T45, CO-OP3, CO-OP5 (Sir Prize), CO-OP6, CO-OP7, CO-OP8, CO-OP9, CO-OP10, CO-OP11, CO-OP12, CO-OP13 (Red Free), CO-OP15, CO-OP16, CO-OP17, CO-OP23 (William's Pride), CO-OP24, CO-OP25 (Scarlett O'Hara), CO-OP26, CO-OP28, CO-OP29 (Sundance™), CO-OP43 (Juliet), Enova, Golden Lasa, Geneva Early, HCR26T132, Jerseymac, Lederer, Melba, NewJersey109, NewJersey56, NewJersey88, Raritan, Red Chief, Red Gala, Starking Delicious, Tesaurus, TN00-027-025, TN00-027-079, Vistabella, Wealty Red. Tested apple trees were grafted on diverse rootstocks, according to the cultivar or selection, the tree age ranged from 2 to 25 years. According to plant availability in the collections, one to several plants per genotype were tested (table 1).

From all screened plants leaf midribs and phloem scrapes were collected mainly during the vegetative season and used for nucleic acid extraction with chloroform/phenol or CTAB methods (Prince *et al.*, 1993; Angelini *et al.*, 2001). The molecular tests were performed amplifying the 16S ribosomal gene with AP specific primers and confirming results amplifying rpS3 and nitroreductase genes as well (Bertaccini *et al.*, 2007).

Nucleic acids of the CO-OP12 samples were compared by SSR analysis. A total of 13 SSRs were analysed in PAGE to verify genotype homogeneity.

Results and discussion

The analyses of the 16S ribosomal gene allowed the detection of '*Ca.* P. mali' in a number of asymptomatic plants belonging to diverse genotypes. Further molecular characterization of '*Ca.* P. mali' strains on rp and nitroreductase genes detected mainly rpX-A/AT-2 type phytoplasmas (table 1). This method distinguish up to 4 different pathogen types (Martini *et al.*, 2007): only phytoplasmas belonging to subgroups rpX-A, and rpX-D were identified.

Results summarized in table 1 clearly indicate that asymptomatic trees can be AP infected as well. Five out of eight Brina trees and four out of seven Tesaurus

Table 1. Occurrence of '*Ca.* P. mali' (infected plants to total tested plants) in asymptomatic apple trees belonging to cultivars or selections maintained in Pergine Valsugana.

Cultivar or selection	Age (years)	Rootstock(s)	no. infected/tested plants	rp/nitroreductase type
Brina	10	MM16	5/8	rpX-A/AT-2 and rpX-D/AT-1
CLR 13 T 45	11	MM106	1/1	nd
CO-OP group	11-25	M9, M26, MM106	5/34	nd
Enova	4-8	M9, Pajam 2	2/11	nd/AT2
Geneva Early	17	M26	0/1	-
Golden Lasa	5	Pajam 2	1/3	rpX-A/nd
HCR 26 T 132	25	M26	0/1	-
Jerseymac	25	M26	0/1	-
Lederer	25	M26	0/1	-
Melba	25	M26	0/1	-
New Jersey group	12	M9	0/3	-
Raritan	25	M26	0/1	-
Red Chief	3	M26	1/1	rpX-A/AT2
Red Gala	2	M9	2/2	rpX-A/AT2
Starking Deliciuos	10	MM106	1/1	rpX-A/AT2
Tesaurus	8-24	M26, PAJAM 2	4/7	rpX-A/nd
TN00-027-025	2	Pajam 2	0/1	-
TN00-027-079	2	Pajam 2	0/1	-
Vistabella	25	M26	0/1	-
Wealty Red	25	M26	0/1	-

trees resulted infected by '*Ca*. P. mali'. For the group of CO-OP trees most of the tested cultivars or selections proved to be not infected by AP.

Interestingly, all negative genotypes in this group showed a fairly complex pedigree including selections from the New Jersey Agricultural Experimental Station (USA). To this regard, it is remarkable that all the trees tested from New Jersey selections resulted negative to AP (table 1). Inside the CO-OP group, selection CO-OP12 appears to be the most promising, since out of 34 plants tested only one was positive to AP in only one out of the three tests performed during the growing season.

To verify plant labelling, SSR DNA analysis was performed, but CO-OP12 samples did not show any polymorphism with the markers employed. Therefore, due to the high level of polymorphisms in apple, genotype mislabelling can be excluded, and the detection of '*Ca*. P. mali' in this CO-OP12 plant sample must be further verified for reliability.

Acknowledgements

Research carried out under the Project granted by the Italian Ministery of Agriculture and Forestry "Controllo delle infezioni da fitoplasmi nel melo e ricerca di fonti di tolleranza e/o resistenza alla ticchiolatura mediante tecniche di biologia molecolare". Paper n. 6.

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