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Molecular investigation on the genetic polymorphism of '*Candidatus* Phytoplasma prunorum' detected in plum and apricot fruit trees

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Abstract

Plum and apricot samples were collected from plants showing symptoms resembling those caused by phytoplasma and grown in different orchards localized in Central and Southern Italy. Samples resulted positive for the presence of '*Candidatus* Phytoplasma prunorum' by molecular analysis. A further genomic investigation on the non-ribosomal DNA region encoding the elongation factor Tu (*tuf* gene) allowed to differentiate a new molecular variant of the phytoplasma in plum samples collected in Calabria region.

Key words: Stone fruit, molecular variant, phytoplasma, ESFY, *tuf* gene.

Introduction

'*Candidatus* Phytoplasma prunorum', (ribosomal group 16SrX-B) is the causal agent of different diseases of stone fruits (Lorenz *et al.,* 1994), described also as European stone fruit yellows (ESFY). The disease was described for the first time on Japanese plum (*Prunus salicina*) in Italy (Goidanich, 1933) and it is now spread in all Italian fruit tree growing regions (Pilotti *et al*., 1995; Del Serrone *et al*., 1998; Carraro *et al*., 2002; Marcone *et al*., 2002).

Symptoms resembling this disease were frequently individuated in the last years in many stone fruit orchards of central and southern Italy. Rolling of leaves and discoloration on single branches develop rapidly into leaf chlorosis and, sometime, necrosis and dieback are observed in the same season.

Field surveys were performed in different Italian regions (Latium, Molise and Calabria) to confirm the presence of phytoplasma. Furthermore to improve the phytoplasma characterization, a molecular investigation was performed on a genome region less conserved than the ribosomal genes in order to evaluate the possible presence of molecular variants.

Materials and methods

Different orchards were monitored during the summer and leaf samples were collected from plum and apricot trees showing symptoms resembling phytoplasma disease. Total DNA extracted from leaf midribs (Marzachì *et al*., 1999) was analyzed by molecular techniques. Direct PCR with the universal primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) was followed by nested-PCR with the universal primers pairs R16F2/R2 (Lee *et al*., 1995) and 16R758F/M23Sr (Gibb *et al.,* 1995; Padovan *et al*., 1995). The phytoplasma identification was obtained by RFLP analysis of amplicons with *Rsa*I and *Taq*I endonuclease, respectively.

A genomic investigation on the non-ribosomal DNA region encoding the elongation factor Tu (*tuf* gene) was carried out in order to molecular characterize the detected phytoplasma. This genome region is, in fact, less conserved than 16S rRNA gene and allows revealing the presence of genetic variability within phytoplasma subgroups. Specifically, primer pairs for initial and nested amplification were designed on *tuf* gene sequence - tuf1f/tuf1r, tuf2f/tuf2r- (not published) and RFLP analysis of relative amplicons was performed with *Nla*III restriction enzyme.

Results

DNA from all symptomatic samples was amplified in nested PCR performed with the universal primers. The RFLP analysis with *Rsa*I endonuclease of R16F2/R2 amplified products and with *Taq*I endonucleases of 16R758F/M23Sr amplicons confirmed the presence of '*Ca.* P. prunorum'.

The designed primers pairs allowed to amplify a *tuf* gene fragment of the expected size. RFLP analysis of relative amplicons with *Nla*III restriction enzyme revealed the presence of two distinct restriction profiles. The first pattern, named 'a', was identical and not distinguishable from those obtained from '*Ca.* P. prunorum' reference strain. The second one, named 'b', was identified only in Calabria samples on the majority of tested plum varieties (12 out of 16) (table 1).

Discussion

The infection of stone fruits with phytoplasmas is known to be wide spread in most growing areas in Italy. Their incidence of infection can differ considerably according to different factors as environmental conditions, cultivar sensitivity, sanitary status of propagation material, agronomic practices and also molecular variability

Table 1. '*Ca.* P. prunorum' isolates identified in plum and apricot samples collected in infected monitored orchards.

Region	Specie	Variety	RFLP Tuf pattern
Latium	Plum	TC Sun	Type a
Latium	Plum	President	Type a
Latium	Plum	Friar	Type a
Latium	Plum	Angeleno	Type a
Latium	Plum	Black Star	Type a
Latium	Plum	Black Diamond	Type a
Latium	Apricot	Pisana	Type a
Latium	Apricot	Portici	Type a
Latium	Apricot	Aurora	Type a
Latium	Apricot	Noemi	Type a
Molise	Apricot	Sancastrese	Type a
Calabria	Plum	TC Sun	Type b
Calabria	Plum	Durado	Type b
Calabria	Plum	Friar	Type b
Calabria	Plum	Green Sun	Type a
Calabria	Plum	Larry Ann	Type b
Calabria	Plum	Original Sun	Type b
Calabria	Plum	October sun	Type a
Calabria	Plum	Bella di Barbiano	Type b
Calabria	Plum	Black Sun	Type a
Calabria	Plum	Black Gold	Type b
Calabria	Plum	Black Diamond	Type a
Calabria	Plum	Black Amber	Type b
Calabria	Plum	Beauty Sun	Type b
Calabria	Plum	Autumn Giant	Type b
Calabria	Plum	Angeleno	Type b
Calabria	Plum	California Blu	Type b

of the pathogen. In this work the presence of a genetic polymorphism within the '*Ca.* P. prunorum' was observed by a molecular investigation of the t*uf* gene. Compared to the genomic profile of the '*Ca.* P. prunorum' reference strain, a new molecular variant was identified in plum samples coming from Calabria Region.

The fact that the same varieties analyzed in different regions resulted infected by the two isolates leads to the conclusion that the genomic characteristics of the hosts do not interfere in the distribution of different phytoplasma types.

More work is in progress to characterize a large number of plum and apricot isolates by PCR and RFLP techniques to better investigate the molecular variability of this phytoplasma and the geographical distribution of the two isolates.

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