Assessment of susceptibility to European stone fruit yellows phytoplasma of new plum variety and five rootstock/plum variety combinations

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Abstract

Two separate experiments were carried out to assess the plum susceptibility to infection by European stone fruit yellows phytoplasmas during a five years period. Commercial varieties/cultivars and new selections grafted on Myrabolan 29C were evaluated in at least two plots of four plants each. Visual inspection and PCR/RFLP identification of phytoplasmas detected an increasing phytoplasma presence in both symptomatic and asymptomatic plants. Eight Japanese plum selections showed ESFY symptoms or pathogen presence in the 50% of the plants and nine selections showed ESFY infection in 20% of the plants. Only nine selections showed absence of both symptoms and pathogen. Although the European selections/cultivars were not symptomatic, plants belonging to six of these cultivars were positive for phytoplasma infection. The evaluation of cultivar/rootstock combinations indicate phytoplasma presence from the first year after plantation on. Two of the rootstocks seem to induce a delay in symptoms appearance and cultivar T.C. Sun resulted to be the most susceptible to the disease independently from the rootstock employed.

Keywords: Japanese plum, European plum, European stone fruit yellows phytoplasmas, resistance, diseases

Introduction

In the last thirty years an increasing presence of European stone fruit yellows phytoplasma (ESFY, '*Candidatus* Phytoplasma prunorum') (Seemüller and Schneider, 2004) on Japanese plum (*Prunus salicina*) was observed in commercial orchards in several European regions (Giunchedi et al., 1978; Desvignes and Cornaggia, 1982; Dosba et al., 1991; Torres et al., 2004). *Prunus* rootstocks (Jarausch et al., 1998) as well as wild *Prunus* species, e.g. *Prunus spinosa* and *P. cerasifera* (Carraro et al., 2002) and cherry (*Prunus avium*) (Paltrinieri et al., 2001) were also reported as infected by these phytoplasmas. In recent years ESFY phytoplasma has been detected in other wild plants such as *Rosa canina*, *Celtis australis*, *Fraxinus excelsior* (Jarausch et al., 2001), and grapevine in Hungary (Varga et al., 2000) and in Serbia (Duduk et al., 2004). The production losses can reach 40% in Japanese plum (Poggi Pollini *et al.*, 1995; Pastore et al., 1999) due to the lack of sanitary control of propagation materials together with the presence of the ESFY-specific vector, *Cacopsylla pruni* (Carraro et al., 1998b) in the orchards.The most evident symptoms on Japanese plum consist of black phloem and leaf malformations such as excessive elongation and upward rolling. European plum cultivars are usually infected only in a latent way (Carraro et al., 1998a). Diseased plants start producing flowers during mild winters, and show then undersized leaves with very little fruit production. The disease usually starts from a branch, affecting within 2-3 years all the plant; infected plants also show abnormal rootstock proliferation. The correct choice of cultivar/rootstock combination that must be tested for resistance/tolerance to the disease is also very important before distributing new cultivars (Desvignes, 1999). During 2003-2008 a research was carried out to assess the ESFY plum susceptibility under natural conditions of disease spreading by visual inspection coupled with molecular analyses.

Material and methods

Two experiments were carried out to verify susceptibility of about 60 plum varieties/cultivars and new selections (A), and of 5 routinely used rootstock/scion combinations (B). The orchards were located in an ESFY severely naturally infected area of Northern Italy (Paltrinieri et al., 2004). Monitoring by visual inspection and PCR/RFLP assays was carried every year out from August to October to verify phytoplasma presence/identity in all plants.

Experiment A: Japanese and European plum varieties and selections grafted into Myrabolan 29C (Table 1 A and B) were evaluated in at least two plots with four plants each.

Experiment B: Three plum commercial cultivars TC Sun, Fortune and Angeleno were grafted on the five rootstocks 'Adesoto 101', 'Ishtara-Ferciana', 'GF 677', 'Montclair-Chanturgue' and 'Myrabolan 29/C'; four plants per each combination per at least two plots were employed.

Tab. 1 A. Results of ESFY phytoplasma identification in Japanese plum cultivars/varieties and selections. B. Results of ESFY phytoplasma identification in European plum varieties/cultivars and selections. In both cases plants were grown in a field located in a severely ESFY naturally infected area.

Japanese plum cultivars and selections		
Table A	**Symptoms**	**n. of ESFY infected plants/n. tested plants**
Anne Gold	yes	2/4
Aphrodite	yes	1/4
Black Glow	yes	1/3
Black Sunrise	yes	3/4
Black Top	no	2/4
Bragialla	no	0/4
Brarossa	no	0/4
Carmen Blu	yes	2/4
Dofi Sandra	yes	0/3
Early Fortune	yes	1/4
Fortune	no	0/4
Gaia	no	1/4
Golden Plumza	yes	1/4
Obilnaja	no	1/4
Red Noble	yes	4/4
Ruby Crunch	no	0/3
Shiro	yes	1/4
Dofi selections (Florence University)		
89.024.004	yes	2/4
89.024.029	no	1/4
89.028.047	no	1/4
89.030.010	no	1/4
89.030.020	no	0/4
89.030.030	yes	1/4
89.030.031	no	0/4
89.036.131	no	0/4
CRA Forli' selections		
IFF on 219	yes	4/4
IFF on 221	yes	4/4
IFF on 260	no	0/4
IFF on 268	no	3/3
IFF on 271	no	0/4

European plum cultivars and selections		
Table B	**Symptoms**	**n. of ESFY infected plants/n. tested plants**
Bellamira	no	0/4
Capitana	no	0/4
Elena	no	0/4
Felsina	no	0/4
Grossa Di Felisio	no	0/4
Jojo	no	0/4
Liablu	no	0/4
Maria Novella	no	0/4
Presenta	no	0/4
President	no	0/4
Rheingold	yes	1/4
Stanley	no	0/4
Tegera	no	0/4
Tipala	no	0/4
Top 2000	no	0/4
Topend Plus	no	0/4
Topfive	no	0/4
Topgigant Plus	no	0/4
Tophit	no	0/4
Tophit Plus	no	0/4
Topking	no	0/4
Topstar Plus	no	0/4
Valcean	yes	3/4
Valerie	yes	1/4
Victory	no	0/4
Agri 2000 selections		
N. 8	no	0/4
N. 10	no	0/4
Hohenheim University selections		
N. 1218	no	0/4
N. 1446	no	0/4
N. 1462	no	0/4
N. 1464	no	0/4
N. 1468	no	0/4
N. 1474	no	1/4
N. 1632	no	0/4
N. 3018	yes	3/4
N. 3217	no	0/4
N. 4913	no	0/4

Molecular analyses: DNA was extracted from fresh leaf midribs and phloem by a chloroform/phenol procedure (Prince et al., 1993). PCR assays were carried out on the nucleic acid samples diluted in TE buffer [10 mM Tris-HCl, 1 mM EDTA (pH 8.0)] to give a final concentration of 20 ng per µl, in total 25 µl reaction mixtures under the conditions described by Schaff et al. (1992). Nested PCR reactions were performed under the same conditions, using as template the products of the previous amplification diluted 1: 30 with sterile water. Positive control samples were DNAs extracted from periwinkle plants infected by phytoplasma strains from the micropropagated collection of DiSTA (University of Bologna), in particular GSFY1, GSFY2 (subgroup 16SrX-B), PD (subgroup 16SrX-C) and AP (subgroup 16SrX-A) were employed.

Phytoplasma primers P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1995) and R16F2/R2 (Lee et al., 1995) amplifying fragments internal to each others in the 16S ribosomal gene were used, respectively in direct and nested PCR. Samples were further tested in nested PCR with primers R16(X)F1/R1 specific for 16SrX (apple proliferation) group (Lee et al., 1994; 1995). Samples with the reaction mixture devoid of DNA template were included in each experiment as negative controls. PCR products were subjected to electrophoresis in a 1% agarose gel and visualised by staining with ethidium bromide and UV illumination.

Three to six µl of PCR products were digested using *Mse*I, *Ssp*I and *Rsa*I restriction enzymes at 37°C for at least 16 hours following the instructions of the manufacturer (Fermentas, Vilinius, Lithuania). The restriction patterns were then compared with those of reference strains after electrophoresis through a 5% polyacrylamide gel in 1X TBE buffer followed by staining with ethidium bromide and visualization under an UV transilluminator.

Results and discussion

Experiment A: After five years 8 Japanese plum selections showed ESFY symptoms or pathogen presence in 50% of the plants and nine selections showed ESFY infection in 20% of the plants. Only 9 selections showed absence of both symptoms and pathogen (Table 1A). None of the European selections/cultivars employed was symptomatic, however plants belonging to 6 cultivars were positive for phytoplasma presence (Table 1B). The comparison of symptom expression and results from PCR/RFLP analyses indicates that selections IFF on 221 and IFF on 219, are very susceptible to ESFY infection; while selection IFF on 268 resulted to be phytoplasma infected even if asymptomatic. A medium level of susceptibility i.e. pathogen or symptom presence starting from the second year after plantation in 50% of the plants, was shown by Anne Gold, Black Sunrise, Black Top, Carmen Blue, Dofi Sandra, Gaia, Obilnaja and Dofi 89.024.004 among the Japanese cultivar and virieties. Less susceptible were Shiro, Black Glow, Dofi 89.030.030, 89.024.029, 89.028.047, 89.030.010, Aphrodite, Early Fortune, and Golden Plumza (one infected plant per cultivar). Varieties Bragialla, Brarossa, Fortune, Ruby Crunch and selections Dofi 80.030.020, 89.030.031, 89.030.131 and IFF on 260 and on 271 were always negative to both symptom presence and molecular analyses. Some European plum cultivars or selections such as Rheingold, Presenta, Valerie, Valcean, 3018 and 1474 were positive to the ESFY presence, however only Rheingold, Valerie, Valcean and selection 3018 showed symptoms possibly related to phytoplasma presence.

Experiment B: The majority of the cultivar/rootstocks combinations showed phytoplasma symptoms and were positive to the PCR/RFLP analyses from the first year after plantation. Two of the rootstocks induced a delay in symptoms appearance indicating some resistance to ESFY in Japanese plum could be present, but only for one-two years after plantation. Intermediate/high susceptibility was detected in varieties grafted on 'GF 677', 'Adesoto 101' and 'Montclair-Chanturgue' showing plants with severe leaf symptoms and reduced growth; while scions grafted on 'Myrabolan 29/C' were efficiently vegetating and growing in spite of the presence of symptoms in the leaves. TC Sun was the most susceptible cultivar to ESFY phytoplasmas in all tested rootstocks; cultivars grafted on 'Ishtara-Ferciana' showed symptoms only after four years from plantation (Figs. 1 and 2) indicating less susceptibility to ESFY infection. The molecular analyses carried out were very important to identify the latently infected combinations that are the most dangerous allowing the pathogen to survive long time in orchards causing recurrent epidemic outbreaks.

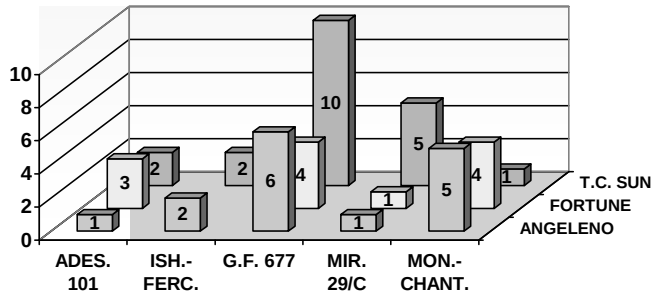

Fig. 1 Number of infected plants for the different cultivar/rootstock combinations

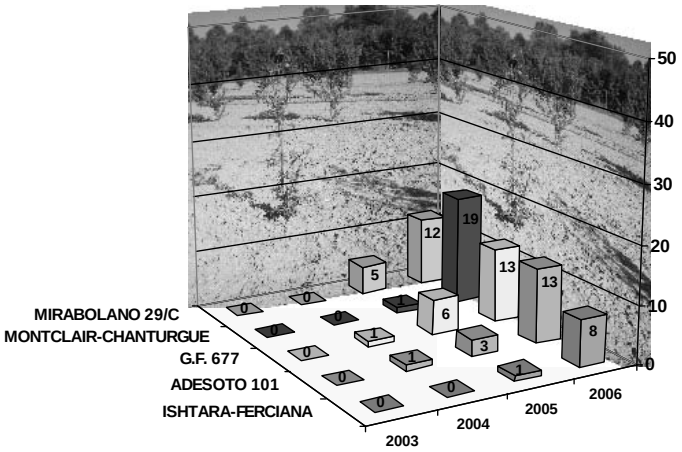

Fig. 2 Number of symptomatic plum plants per year grouped by rootstock.

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