Bulletin of Insectology ***64*** *(Supplement): S273-S274, 2011*
ISSN 1721-8861

Response of apple proliferation-resistant *Malus sieboldii* hybrids to multiple infections with latent apple viruses

Anna Maria CICCOTTI, Claudia BISOGNIN, Ivana BATTOCLETTI, Marco DEROMEDI, Paola BRAGAGNA, Mauro FILIPPI
Research and Innovation Centre - Fondazione E. Mach, 1, 38010 San Michele all'Adige, Trento, Italy

Abstract

Apple proliferation (AP) is the most important phytoplasma-associated disease affecting apple in Europe. The failure in controlling this disease by standard means strongly increased the importance of adopting resistant genotypes. About 6000 seedlings were obtained from a breeding programme crossing *M. sieboldii,* donor of resistance to AP, with standard apple rootstocks (M9 mainly) as donor of agronomic value. Resistance screening showed that the trait was inherited to the progenies and trials are in progress to test the agronomic value of these genotypes. In an additional trial, the response of AP-resistant genotypes to a superinfection with different latent apple viruses was investigated. For this, *M. sieboldii*-derived first and second generation hybrids were analysed. In summer, three repetitions for each genotype were inoculated with *apple chlorotic leaf spot* (ACLSV), *apple stem grooving* (ASGV) and *apple stem pitting* (ASPV) virus. The two following springs after infection, the presence of the viruses was assessed by ELISA test and virus-specific symptom recording on young leaves. In parallel, the reaction of the plants to infections with Trentino strains of '*Candidatus* Phytoplasma mali' was evaluated. AP-susceptible *Malus* x *domestica* genotypes were considered as controls. The results confirmed an incidence of the viral infections on *Malus sieboldii* as it was reported in the past. However, the *M. sieboldii* hybrids showed a high variability of response ranging from no viral symptoms to severe symptoms. Nevertheless, highly phytoplasma-resistant genotypes which showed no presence of viral superinfections could be identified in these experiments.

Key words: '*Candidatus* Phytoplasma mali', *apple stem grooving* virus, *apple stem pitting* virus, resistance screening, breeding.

Introduction

Apple proliferation (AP) is one of the most important phytoplasma diseases in Europe that causes considerable economic losses. It is transmitted by grafting, insect vectors and root bridges (Ciccotti *et al*., 2007). The failure in controlling this disease by standard means strongly increased the importance of adopting resistant genotypes. Previous work indicated that, due to the colonization behavior of the associated agent, the disease can be controlled by the use of resistant rootstocks (Seemüller *et al.,* 1984). While extensive screening revealed no satisfactory resistance in established rootstocks (Kartte and Seemüller, 1991), substantial levels of resistance were identified in experimental rootstocks derived from crosses of the apomictic species *Malus sieboldii* and genotypes of *M.* x *domestica* and *M.* x *purpurea* (Bisognin *et al.,* 2008a and b; Seemüller *et al.,* 2008).

As these experimental rootstocks had poor agronomic values, a breeding programme was started ten years ago in order to develop commercial AP-resistant apple rootstocks exploiting the natural resistance found in *Malus sieboldii* (Bisognin *et al.,* 2009). Resistance screening showed that the trait was inherited by the progenies and trials are in progress to test the agronomic value of these genotypes (Jarausch *et al*., 2010). Moreover, some apomictic rootstocks budded with a virus-contaminated scion source revealed great differences in susceptibility to such viruses that include *apple chlorotic leaf spot virus* (ACLSV), *apple stem pitting virus* (ASPV) and *apple stem grooving virus* (ASGV) (Seemüller *et al*., 2008). In the present study, the response of different *Malus sieboldii* hybrids to infection with three different latent viruses was investigated and compared with phytoplasma resistance of these genotypes to two Trentino strains of '*Candidatus* Phytoplasma mali'.

Materials and methods

Healthy one-year-old micropropagated plants of *M. sieboldii*-derived first and second generation hybrids, *M. sieboldii*, 4551, D2212, H0909, H0801 o.p., Gi 477/4 o.p., C1907 o.p, 4551 o.p. (Ciccotti *et al.*, 2008) and selected hybrids obtained from the crosses 4551xM9, D2212xM9, H0909xM9 and M9xD2212 (for details see Bisognin *et al*., 2009), were inoculated in pots *ex vitro* during summer 2008. Some AP-susceptible genotypes were taken as control.

In a first experiment three replicates for each genotype were separately inoculated by chip budding with *apple chlorotic leaf spot* (ACLSV), *apple stem grooving* (ASGV) and *apple stem pitting* (ASPV) virus. In a second experiment three replicates for each genotype were contemporary inoculated with the three viruses to evaluate the reaction of the plants to superinfection. Trials were conducted in an insect-proof screenhouse. In spring 2010 ELISA test was used to evaluate the presence of the viruses and symptoms were recorded on young leaves. Symptom incidence of the viruses was evaluated as follows: 0 = no symptoms, x = low incidence, xx = moderate incidence, xxx = high incidence.

The same genotypes were evaluated in a parallel experiment for AP resistance. *Ex vitro* plants were inoculated by grafting with phytoplasma infected scions with two '*Ca.* P. mali' strains PM6 and PM11 isolated in Trentino, Northern Italy. Three repetitions for each genotype-strain combination were performed. The second autumn after inoculation, phytoplasma infection was evaluated and expressed by a disease index based on incidence of specific symptoms such as enlarged stipules, witches brooms, foliar reddening, stunting (index values ranged from 0 = no symptoms to 4 = high presence of symptoms). In the same period '*Ca.* P. mali' concentration in the roots was also evaluated by real time quantitative PCR (data not shown).

Results and discussion

In the first experiment single infections with the latent apple viruses ACLSV, ASGV and ASPV were difficult to evaluate as more than 50% of the plants were not infected as assessed by ELISA. In contrast, the multiple infections of the *M. sieboldii* hybrids with all three viruses yielded an incidence of the viral symptoms ranging from no to severe symptoms. Indeed, sensitivity of apomictic rootstocks to latent apple viruses was already observed by Schmidt (1988) as stunting and chlorosis. Seemüller *et al.* (2008) observed a poor development and stunting of *M. sieboldii* and 4,551 seedlings inoculated accidentally with both, phytoplasma and latent viruses. Our results showed that plants of *M. sieboldii* and 4,551 selections were slightly to moderate affected by the multiple presence of viruses alone. In contrast, D2212 which was less affected in the work of Seemüller *et al.* (2008) showed no symptoms of virus infections and behaved as the tolerant *M.* x *domestica* control M9. The same was observed for plants of the apomictic selections like C1907 and Gi 477/4 which were originally derived from open pollination. Interestingly, the sensitivity to latent apple viruses was expressed very heterogeneously in the progeny of the crosses made with D2212, H0909 and 4551. The progeny genotypes showed either no viral symptoms or were much more severely affected as the parental *M. sieboldii*-derived genotypes.

The objective of the work to find a rootstock resistant to AP and tolerant to the latent apple viruses was achieved by apomictic genotypes like D2212 and C1907 o.p. as well as by some selected progeny of the breeding programme as tested here. These genotypes showed no or only mild symptoms upon inoculation with the Trentino strains of '*Ca.* P. mali' and exhibited no viral symptoms after multiple infections.

These finding should be confirmed in further trials in which breeding genotypes will be used as rootstocks of commercial varieties in order to follow the influence of virus infections also in the production of the plants. After this step the response to infection will be completely understood.

Acknowledgements

This work was supported by the project SMAP funded by the Autonomous Province of Trento, and carried out in the frame of COST action FA0807 "Integrated Management of Phytoplasma Epidemics in Different Crop Systems".

References

Bisognin C., Schneider B., Salm H., Grando M. S., Jarausch W., Moll E., Seemüller E., 2008a.- Apple proliferation resistance in apomictic rootstocks and its relationship to phytoplasma concentration and simple sequence repeat genotypes.- *Phytopathology*, 98: 153-158.

Bisognin C., Ciccotti A. M., Salvadori A., Moser M., Grando M. S., Jarausch W., 2008b.- *In vitro* screening for resistance to apple proliferation in *Malus* species.- *Plant pathology*, 57(6): 1163-1171.

Bisognin C., Seemüller E., Citterio S., Velasco R., Grando M. S., Jarausch W, 2009.- Use of SSR markers to assess sexual vs. apomictic origin and ploidy level of breeding progeny derived from crosses of apple proliferation-resistant *Malus sieboldii* and its hybrids with *Malus* x *domestica* cultivars.- *Plant Breeding*, 128: 507-513.

Ciccotti A. M., Bianchedi P., Bragagna P., Deromedi M., Filippi M., Forno F., Mattedi L., 2007.- Transmission of '*Candidatus* phytoplasma mali' by root bridges under natural and experimental conditions.- *Bulletin of insectology*, 60(2): 387-388.

Ciccotti A. M., Bisognin C., Battocletti I., Salvadori A., Herdemertens M., Jarausch W., 2008.- Micropropagation of apple proliferation-resistant apomictic *Malus sieboldii* genotypes.- *Agronomy Research*, 6(2): 445-458.

Jarausch W., Bisognin C., Grando S., Schneider B., Velasco R., Seemüller E., 2010.- Breeding of apple proliferation resistant-rootstocks: where are we?- *Petria*, 20(3): 675-677.

Kartte S, Seemüller E, 1991.- Susceptibility of grafted *Malus* taxa and hybrids to apple proliferation disease.- *Journal of Phytopathology*, 131: 137-48.

Schmidt H., 1988.– Criteria and procedures for evaluating apomictic rootstocks for apple.- *HortScience*, 23: 104-107.

Seemüller E., Kunze L., Schaper U., 1984.- Colonization behaviour of MLO, and symptom expression of proliferation-diseased apple trees and decline-diseased pear trees over a period of several years.- *Journal of Plant Disease and Protection*, 91: 525-532.

Seemüller E., Moll E., Schneider B., 2008.- Apple proliferation resistance of *Malus sieboldii*-based rootstocks in comparison to rootstocks derived from other *Malus* species.- *European Journal of Plant Pathology*, 121: 109-119.

Corresponding author: Anna Maria Ciccotti (e-mail: annanaria.ciccotti@iasma.it) Research and Innovation Centre - Fondazione E. Mach, 1, 38010 San Michele all'Adige, Trento, Italy.