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

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**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 assessed 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 infections of the *Malus* species. In contrast, the multiple infections derived from crosses of apple proliferation-resistant *Malus sieboldii* and its hybrids with *Malus* taxa and hybrids to apple proliferation disease.- *Journal of Plant Disease and Protection*, 98: 153-158.


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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”.

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