

## Breeding of rootstocks resistant to apple proliferation disease

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### Abstract

Apple proliferation (AP) is caused by the wall-less bacterium *Candidatus Phytoplasma mali* and is spread by psyllids. Previous work indicated that, due to the colonization behavior of the causal agent, the disease can be controlled by the use of resistant rootstocks. However, extensive screening revealed no satisfactory resistance in established rootstocks. In contrast, 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*. However, trees on these hybrids are more vigorous and less productive than trees on standard stock M 9. For this reason, a program was initiated to reduce vigor and improve yield by crossing and backcrossing *M. sieboldii* and its apomictic hybrids with M 9 and other dwarfing stocks. From 2001 through 2006 a total of 36 crosses were made. However, only 23 progenies consisted of a substantial number of seedlings while the other crosses largely failed due to pollen incompatibility. The 3,500 seedlings obtained were DNA-typed using codominant SSR markers to distinguish apomicts and recombinants in the progenies. A total of 1,800 seedlings consisting of all recombinants and a representative number of apomicts were screened for AP resistance by graft inoculation followed by observation in the nursery and under commercial growing conditions. Several progenies showed a good inheritance of resistance. In two of them (4608 x M 9 and D2212 x M 9) more than 50% of the individuals never developed symptoms.

Keywords: *Candidatus Phytoplasma mali*, *Malus sieboldii*, SSR analysis

### Introduction

Apple proliferation (AP) is caused by the wall-less bacterium *Candidatus Phytoplasma mali*, and is a serious disease in several major apple-growing areas of western and central Europe. The disease is difficult to control. The most often recommended means, phytosanitary measures and insecticide treatments against the insect vectors, are often not fully satisfactory. The most promising approach to control AP would be the use of resistant plants.

Previous work revealed that phytoplasma colonization in the aerial parts of the tree is subject to seasonal fluctuation. *Ca. P. mali* is eliminated in the top during winter and cannot be detected by fluorescence microscopy in early spring. Transmission by grafting aerial tissue was not possible in this period (Seemüller et al., 1984). Elimination of the pathogen in the stem is due to the degeneration of phloem sieve tubes the functional status of which the pathogen is depending on. Overwintering occurs in the roots where intact sieve tubes and phytoplasmas are present throughout the year (Schaper and Seemüller, 1982). From the roots the stem may be recolonized in spring when new phloem is being formed (Schaper and Seemüller, 1984). This fluctuation in the colonization pattern of the stem has been confirmed recently for Northern Italian (Trentino) conditions (Pedrazzoli et al., 2008). The overwintering of the pathogen in the roots has led to the presumption that growing scion cultivars on resistant rootstocks can prevent the disease or reduce their impact.

There was little information on the influence of apple rootstocks on the occurrence of AP. Resistance screening of *Malus x domestica* rootstocks revealed that there is no satisfactory resistance in the established stocks. However, natural resistance to AP was detected in experimental apomictic rootstocks with *M. sieboldii* in their parentage (Seemüller et al., 1992). These rootstocks, which develop seeds that are genetically identical to the mother, have been developed in the 1950s and 1960s for easy propagation by seeds, virus-free plants, better anchorage and higher resistance to some fungal and bacterial diseases such as crown rot, apple scab, powdery mildew and fire blight (Schmidt, 1988). However, these apomictic rootstocks did not succeed in European apple cultivation because the trees grown on them were mostly more vigorous and less productive than trees on standard stock M 9. Year long field trials carried out with these *M. sieboldii*-derived rootstocks under natural and experimental infection conditions confirmed the resistance which is characterized by the absence of symptoms and a highly reduced concentration of the pathogen in the plant. These field trials demonstrated the feasibility of the adopted resistance strategy by preventing the development of aerial symptoms including undersized fruits in susceptible cultivars (Bisognin et al., 2009; Seemüller et al., 2008).

Based on these findings a breeding program was started in 2001 which aims to improve the agronomic value of the *M. sieboldii*-derived rootstocks.

## Material and methods

Parentage and ploidy levels of AP-resistant apomictic *M. sieboldii* hybrids used in the crosses made from 2001-2006 are listed in Table 1. Details on pollination, seedling growing and microsatellite or simple sequence repeat (SSR) analysis to identify recombinant seedlings, were as described by Bisognin et al. (Bisognin et al., 2009). Recombinant genotypes were screened by graft-inoculating potted seedlings in the first or second year of growth using scions from Golden Delicious trees showing witches'-broom symptoms. Evaluation of resistance was carried out in a three-step procedure. Following inoculation in July or August, the plants were kept in pots until the spring of the following year. In this period, highly susceptible plants declined or were severely stunted. Such plants were discarded whereas the lightly affected and unaffected plants were transplanted to the nursery where they were observed for two or three years. Plants that never showed symptoms or recovered from disease in the nursery phase were considered to be resistant and were transplanted at standard spacing for further evaluation for resistance and pomological traits under commercial growing conditions. Quantitative real-time PCR for *Ca. P. mali* quantification was carried out as described (Seemüller and Schneider, 2007).

**Tab. 1** Parentage and ploidy level of AP-resistant apomictic *M. sieboldii* hybrids used in breeding from 2001-2006

<i>M. sieboldii</i> Hybrid	Parentage	Ploidy level
4551	<i>M. x domestica</i> cv Laxton's Superb x <i>M. sieboldii</i>	3n
4608	<i>M. purpurea</i> cv Eleyi x <i>M. sieboldii</i>	3n
C1907	4608, open pollinated	4n
D2118	4556, open pollinated	4n
D2212	( <i>M. x domestica</i> cv Laxton's Superb x <i>M. sieboldii</i> ), open pollinated	4n
Gi477/4	4808, open pollinated	4n
H0801	( <i>M. x domestica</i> cv Laxton's Superb x <i>M. sieboldii</i> ) x M 9	4n
H0909	( <i>M. x domestica</i> cv Laxton's Superb x <i>M. sieboldii</i> ) x M 9	4n

## Results and discussion

**Breeding data and SSR categories:** From 2001 through 2006 a total of 36 crosses were made. The AP-resistant parents *M. sieboldii* and the F<sub>1</sub> and F<sub>2</sub> hybrids of *M. sieboldii* listed in Table 1 were crossed mainly with M 9, occasionally with other dwarfing rootstocks P 22, M 27 and Supporter 1, or with the scion cultivar Gala in order to reduce vigor and improve productivity. Of the crosses made, 23 yielded a satisfactory number of seedlings. In contrast, crosses with the triploid *M. sieboldii* hybrids 4608 and 4551 as male parents resulted in few or no seeds due to unsuitable pollen properties. Also, tetraploid apomicts such as *M. sieboldii* and hybrid D2212 were often poor pollinators for M 9 and other *M. x domestica*-based genotypes. For these reasons, the resistant apomicts were preferentially used as seed parents.

As expected for apomictic seed parents, SSR analysis revealed that the majority of the plants of most progenies was genetically identical to the mother. However, apomixis is not obligate in *Rosaceae* and additional fertilization following autopollination and cross pollination is possible. Crosses of triploid apomictic seed parents 4608 and 4551 with a diploid pollen parent, such as M 9, resulted in two categories of seedlings, namely motherlike plants and plants having the unreduced maternal genome combined with a recombinant set of alleles from the male parent (in the following referred to as 'hybrid I'). A higher variability in the progeny was obtained in crosses of tetraploid apomicts with M 9 or other diploid *M. x domestica* genotypes as pollen parents. In such cases, two additional combinatorial categories occurred that included full recombinants (recombination of the maternal and paternal genotype, referred to as 'hybrid II') and plants derived from autopollination. Thus, such combinations were preferred in the more recent crosses because they appeared to offer better chances to obtain suitable genotypes for commercial apple growing. The breeding products obtained up to the crosses made in 2006 sum up to a total of more than 3,500 plants. Major successful crosses were 4608 x M 9, 4551 x M 9, H0909 x M 9, H0909 x Supporter 1, D2212 x M 9, Gala x *M. sieboldii*, D2118 x M 9, C1907 x M 9 and H0801 x M 9. In 2007 and 2008 the breeding program was continued by using genotypes derived from the previous cross 4608 x M 9.

**Evaluation of resistance:** Resistance of the progenies obtained from the resistant apomicts was evaluated by rating the various disease symptoms and accumulating the annual values over the entire observation period (Seemüller et al., 2008). This procedure leads to a cumulative disease index (CDI) for each seedling or each progeny. Low CDI values associated with a high percentage of plants that never showed symptoms or only temporarily mild symptoms were only shown by the progenies of the 4608 x M 9 and D2212 x M 9 crosses. Progenies obtained from crosses of other resistant apomicts as seed parents with *M. x domestica* genotypes showed distinctly higher disease values. These higher CDI

values might be at least partially due to the high sensitivity of some genotypes to latent apple viruses (Seemüller et al., 2008).

Preliminary data from the orchard-scale screening are only available for the progenies of the crosses made in 2001 and 2002. From these progenies, a total of 254 trees mostly grown on recombinant rootstocks were transplanted from the nursery to the orchard in 2005 and 2006, respectively. Most of these trees did not develop any symptoms or developed only temporarily mild symptoms such as foliar reddening. Only 9 % of the trees showed moderate symptoms, mainly reduced vigor in combination with foliar reddening. In contrast, similarly inoculated control trees on M 9 exhibited permanently severe symptoms including undersized fruits and strongly reduced vigor. Trees on resistant rootstocks varied considerably in yield and vigor. Phytoplasma concentration in trees on resistant and susceptible rootstocks was determined in progenies of the cross H0909 x M 9 during the screening period in the nursery. RT-PCR analysis of inoculated trees revealed that the titer in the roots of moderately to severely affected trees ( $CDI \leq 1$ ) is 15 to 23 times higher than in trees showing no or mild symptoms ( $CDI > 1$ ). In the shoots the differences are in the range of 60 in the two symptom categories. These findings are in agreement with the previous finding that the phytoplasma concentration in trees on resistant, *M. sieboldii*-based rootstocks is lower than in trees on susceptible stocks (Bisognin et al., 2008).

## Conclusions

In this breeding project *M. sieboldii* and several apomictic *M. sieboldii*-derived hybrids were crossed with *M. x domestica*-based genotypes, mainly rootstock M 9, in order to reduce vigor and improve yield of trees on apomicts. Screening of this material by graft inoculation revealed segregation of the resistance trait. In crosses of resistant apomicts that were tolerant to latent apple viruses more than 50 % of the recombinant progeny showed a high level of AP resistance. A considerable number of AP-resistant and virus-tolerant seedlings were also obtained from virus-sensitive apomictic parents. Promising seedlings are being screened for pomological suitability under commercial growing conditions. In these field tests, a considerable variability in vigor and productivity within recombinant progeny plants are being observed. The inheritance of AP resistance and the variability in vigor and productivity offer a good chance to select AP-resistant stocks with suitable pomological properties.

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## Literature

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