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Breeding apple proliferation-resistant rootstocks: durability of resistance and pomological evaluation

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Abstract

To develop apple proliferation (AP) resistant rootstocks, a breeding program was initiated in 2001 employing apomictic *Malus sieboldii* and *M. sieboldii*-derived hybrids as donors of the resistance trait and mainly standard apple rootstock *Malus* x *domestica* cv. M9 as donor of agronomic values. Examination of the experimentally inoculated progeny of seven crossings made in 2001 and 2002 over a period of seven to eight years showed that inheritance of resistance differs considerably among the parental lines by yielding between 10 to 55% of resistant offspring. Resistant rootstocks were characterized by poor host properties for the AP phytoplasma. This is evidenced by lower phytoplasma titers in resistant genotypes than in standard stock M9 and by preventing detectable phytoplasma development in the top grafted susceptible cv. Golden Delicious. At the end of the observation period, 80% of the root samples collected from resistant rootstocks tested PCR-negative. Size and productivity of trees grown on resistant rootstocks varied over a wide range. A preliminary pomological evaluation was done on the cv. Golden Delicious which has been grafted as infected scion on the progeny genotypes to test. Comparisons with trees grown on M9 rootstocks indicated that there are genotypes among the offspring examined that fulfill the requirements of commercial apple growing.

Key words: Apple proliferation, apple rootstocks, *Malus sieboldii*, phytoplasma titer, resistance breeding.

Introduction

Apple proliferation (AP) is induced by the wall-less bacterium '*Candidatus* Phytoplasma mali' and is widespread in several major fruit-growing areas in Europe. AP is of considerable economic importance due to its negative effect on tree productivity and fruit quality.

The disease is difficult to control. Because the recommended measures are often not satisfactory, the most promising approach to control AP appears the use of resistant plants. Previous work indicated that, due to the seasonal fluctuation of '*Ca*. P. mali' between stem and roots of infected apple trees, growing the scion cultivars on resistant rootstocks can prevent the disease (Schaper and Seemüller, 1982; Seemüller *et al.*, 1984).

However, examination of many established and experimental rootstocks, which were mostly based on *Malus x domestica*, and a large number of other *Malus* taxa revealed that there is no satisfactory resistance in these groups (Kartte and Seemüller, 1991; Seemüller *et al.*, 1992). Suitable resistance was observed in some experimental rootstock selections derived from the apomictic species *M. sieboldii* (Bisognin *et al.*, 2008; Seemüller *et al.*, 2008).

Because trees on the resistant genotypes were more vigorous and less productive than trees on standard stock M9, a breeding program was initiated to develop resistant rootstocks with satisfactory pomological properties by using *M. sieboldii*-based parental lines from the above screening as donors of resistance and M9 and other dwarfing stocks as donors of pomological values (Bisognin *et al.*, 2008; Bisognin *et al.*, 2009; Jarausch *et al.*, 2007; Jarausch *et al.*, 2010). Here we present results of long-term observations on level and durability of resistance, the relationship of phytoplasma concentration to resistance, and on vigor and productivity of trees grown on selected offspring.

Materials and methods

In 2001 and 2002 the following crossings were made that resulted in a substantial number of offspring (see Bisognin *et al.*, 2009 for details): 4551 (Laxton's Superb x *M. sieboldii*) x M9; 4608 (*M. purpurea* 'Eleyi' x *M. sieboldii*) x M9; H0909 [(Laxton's Superb x *M. sieboldii*) x M9)] x P22; H0909 x M9; *M. sieboldii* x M9; D2212 [(Laxton's Superb x *M. sieboldii*) x o.p.)] x M9; M9 x D2212.

All progenies were grown in pots in the greenhouse. Sets of 5 to 6 locus-specific simple sequence repeats (SSR) markers were employed to distinguish sexually derived seedlings from apomictically derived seedlings (Bisognin *et al.*, 2009). In July, preferentially recombinant seedlings, for comparison also a representative number of motherlike (apomictic) plants, were graft-inoculated with cv. Golden Delicious infected with severe strains of '*Ca.* P. mali'. The following spring, inoculated plants were transplanted to the nursery where they were observed for 2 to 3 years for symptom expression on Golden Delicious. All trees on recombinant seedlings that never developed symptoms or only temporarily mild symptoms such as foliar reddening or enlarged stipules were considered to be resistant and were transplanted for further evaluation under commercial growing conditions. In addition, some trees on

motherlike seedlings were also transplanted. Symptom development and yield were recorded annually for 4 to 5 years. Vigor as expressed by trunk diameter in 40 cm height was determined in fall of 2010.

Quantitative real-time PCR was performed to determine presence and concentration of *'Ca.* P. mali' in inoculated trees as described (Bisognin *et al.*, 2008).

Results and discussion

From the crossings made, approximately 3,000 offspring were obtained. SSR genotyping revealed that the majority of them derived from apomixis. Some 750 seedlings were inoculated of which 535 were recombinant.

Screening during the nursery phase revealed considerable differences in the inheritance of AP resistance of the various apomictic parents used. The best donors of this trait were selections 4608 and D2212. Crossings of these genotypes with M9 yielded 60 to 70% recombinant offspring classified as resistant. In the progenies of the other crossings resistance ranged between 20 and 30%.

At the end of the nursery growing phase, 207 trees on recombinant rootstocks and 47 trees on motherlike rootstocks were selected and transplanted for further evaluation. In the following field observation period, 70 to 80% of the trees on both recombinant and motherlike rootstocks continued to show excellent resistance properties whereas the remaining trees showed, mostly temporarily, mild to moderate symptoms. Only the trees on rootstocks derived from selection H0909 depicted lower values on the persistence of resistance, being in the range of 40%. In contrast to selected trees from resistant parents, all transplanted control trees on M9 rootstocks showed permanently moderate to severe AP symptoms.

Quantitative RT-PCR showed that the phytoplasma titer in *M. sieboldii*-derived resistant rootstocks is usually in the range from 10^4 to 10^6 cells/g phloem. In the roots of M9 stock the titer was 10 to 1,000 times higher. This is confirming previous findings (Bisognin *et al.*, 2008). Accordingly, the phytoplasma titer in roots of severely affected progeny genotypes was found to be about 10 times higher than in offspring that was not or only slightly affected. Furthermore, there is indication that the phytoplasma infection in resistant roots is eliminated or reduced to an undetectable level. Eight years post inoculation, 80% of the root samples collected from resistant stocks tested PCR-negative.

At the end of the observation period the selected trees on recombinant rootstocks differed considerably in size. Most of them were too vigorous for the commercial growing of culinary apples. However, in all progenies recombinant stocks were identified that mediate satisfactory dwarfism to the scion cultivar. E.g., more than 10% of the trees on resistant genotypes of the 4608 x M9 progeny were similar in size to trees on M9.

The correlation of productivity and vigor known from established rootstocks also applies for the selected resistant stocks. Regarding trees on resistant stocks derived from the most successful crossing 4608 x M9, the cumulative yield ranged from 1.6 to 5.0 kg/cm^2 cross section of the trunk. Comparison showed that the average yield of trees on dwarfing stocks was 3.7 kg/cm^2 whereas that of trees on stocks too vigorous for commercial growing was only 2.4 kg/cm^2.

Promising genotypes are currently multiplied by micropropagation for further agronomic evaluation.

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