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Breeding of apple rootstocks resistant to '*Candidatus* Phytoplasma mali'

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Abstract

To obtain apple rootstocks resistant to apple proliferation and suitable to modern fruit growing, 24 cross combinations were performed over a 5-years period using *M. sieboldii* and its hybrids as donors of the resistance trait and standard apple rootstock *M. domestica* genotypes as donors of agronomic values. As the resistance donors had different degrees of polyploidy, not all cross combinations were compatible and produced seeds. Out of 17 cross combinations more than 3,000 individuals were obtained which were analysed by co-dominant SSR DNA markers in order to distinguish non-recombinant, apomictic progeny from recombinant progeny. In total, 13% of all progenies showed a complete recombination of the maternal and paternal genotype and in 25% the unreduced apomictic genotype was recombined with one haploid *M. domestica* genotype. Thus, breeding with the apomictic genotypes could be achieved. All recombinant progeny seedlings were graft-inoculated with '*Candidatus* Phytoplasma mali' to evaluate the resistance behaviour. The inoculated seedlings were maintained in the nursery and apple proliferation symptoms were recorded every year in autumn. Two years after inoculation the roots of the inoculated plants were analysed by PCR for the presence of '*Ca.* P. mali'. A quantitative real-time PCR assay was employed to quantify the phytoplasmas in the different genotypes. The results indicate that the resistance trait can be inherited to the progeny. An agronomic evaluation of the resistant genotypes has now to follow.

Key words: Apple proliferation, *Malus sieboldii*, apomixis, polyploidy, microsatellite analysis, resistance screening, quantitative real-time PCR.

Introduction

Apple proliferation (AP) is a disease widespread in central and southern Europe which causes important economic losses due to undersized fruits with poor taste. All currently grown apple cultivars and rootstocks are susceptible to the disease and no curative treatments are applicable. The causal agent of the disease, the yet uncultured phloem-restricted phytoplasma '*Candidatus* Phytoplasma mali', is naturally spread by psyllid vectors and may also be transmitted through root bridges. The disease can be introduced into the orchards by infected planting material. Due to this different ways of disease spread, control of AP is difficult. Thus, the use of resistant plant material could be the only means to control the disease.

Since 1980, natural resistance towards AP was looked for in hundreds of cultivated, wild and ornamental *Malus* genotypes. Resistance to AP was discovered mainly in the wild apomictic *Malus* species *Malus sieboldii* and *M. sieboldii*-derived hybrids (Kartte and Seemüller, 1991). Crossings of *M. sieboldii* with *M. domestica* were carried out in the 1950s and 1970s in order to obtain apomictic rootstocks for apple amenable to seed propagation (Schmidt, 1964; Schmidt, 1988). However, these rootstocks were not appropriate for modern apple growing due to their high vigour and alternate cropping (Schmidt, 1988). Despite their varying degrees of apomixis and ploidy, these genotypes represent valuable material for new breeding of AP resistant rootstocks of agronomic value.

This natural resistance towards AP can be exploited in a resistance strategy because the phytoplasmas are eliminated in the upper part of the tree, the grafted cultivar, once a year due to the phloem renewal in late winter/early spring (Seemüller *et al*., 1984). The phytoplasmas survive in susceptible rootstocks all over the year because phloem renewal is a continuous process in the root system. Thus, resistant rootstocks could impair this survival and the re-colonisation of the stem in spring.

The objective of the breeding program was therefore to provide a durable solution to AP by introducing the resistance trait into commercial rootstocks.

Materials and methods

The breeding program was based on the resistant genotypes *Malus sieboldii*, its F_1 hybrids 4551, 4608 and its F_2 hybrids C1907, D2218, D2212, H0801, H0909 and Gi477/4 (Schmidt, 1964). The *M. domestica* rootstock cultivars J-TE-F, M9, M27, P22 and Supporter 1 were used as donors for the agronomic values and *M. domestica* Golden Delicious, Gala and Prima as controls.

The breeding was done by pollinating the resistant genotypes with the pollen of the *M. domestica* genotypes and vice versa. Seeds were germinated during winter and seedlings were grown in the greenhouse. In summer they were graft-inoculated with '*Ca.* P. mali' in July. Inoculated plants were observed in the nursery for

symptom expression.

All seedlings obtained in the breeding program were DNA typed using co-dominant SSR markers (Liebhard *et al.*, 2002) to establish their pedigree.

PCR detection of *'Ca.* P. mali' was done using specific primers AP3/AP4 as published by Jarausch *et al.* (1994). Phytoplasma quantification was carried out by quantitative PCR as described by Jarausch *et al.* (2004).

Results and discussion

More than 3,000 seedlings belonging to 17 progenies were obtained from 24 different cross combinations made in the years 2001 – 2005. Some cross combinations yielded no seeds due to poor pollen quality or putative incompatibility. If apomictic genotypes were used as female parent a high yield of seeds was obtained. On the contrary, if pollen from apomictic genotypes was used to pollinate *M. domestica* genotypes only few seeds were produced. Co-dominant SSR markers were employed to identify the recombinants in the progeny. As expected, in 79% of all progenies the marker profile matched completely with the maternal apomictic genotype indicating seed development by apomixes. However, in 13% of the total progenies a complete recombination of maternal and paternal genotype was found. The number of these fully recombinant progeny genotypes varied considerably among the different cross combinations and was highest in crosses with tetraploid apomictic genotypes (*M. sieboldii* and its F_2 hybrids). A second class of recombinants was found in which the unreduced apomictic genotype was recombined with one haploid *M. domestica* genotype. This class of hybrids encountered for 25% of the progeny. In cross combinations with triploid apomictic genotypes (*M. sieboldii* F_1 hybrids) all recombinant progeny felt into this class.

All recombinant progeny was graft-inoculated with '*Ca.* P. mali'-infected Golden Delicious to screen for AP resistance in the progeny. For preliminary screening the inoculated plants were observed for two consecutive years in the nursery. After this period the roots of interesting genotypes were analysed by PCR for phytoplasma presence and by quantitative PCR for phytoplasma concentration. As expected, a variable response towards the phytoplasma infection was observed with the fully recombinant hybrids but also with paternal recombinant hybrids. The majority of the seedlings developed moderate to severe symptoms in the first year post inoculation, In the second year after inoculation susceptible genotypes showed again severe symptoms in autumn whereas resistant genotypes showed no symptoms anymore. These observations were confirmed by qPCR data where lower phytoplasma concentrations were found in the putative resistant genotypes. These data match perfectly with a recent re-evaluation of the resistance of the parental genotypes in a long-year's field trial under high natural infection pressure (Bisognin *et al*., 2007). Resistant genotypes exhibited no or only slight symptoms in the susceptible cultivar and were characterised by a low phytoplasma concentration in the resistant rootstock. Up to two thirds of the recombinant progeny of the best donors of resistance (D2212, 4608, 4551) showed this phenotype indicating that the resistance could be inherited. An agronomic evaluation of these genotypes has now to follow.

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