Breeding apples with durable resistance on the genetic basis of old local varieties

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Abstract

Breakdown of Rvi6 resistance against Venturia inaequalis *accelerated the search for new sources of resistance to apple scab. On the one hand breeders are working with resistance genes from wild malus species, on the other hand genes derived from old local varieties conferring partial resistance have become a focus in recent times. Approaches followed by LVWO Weinsberg show that crossings with old local varieties based on partial resistance need to have a higher number of descendants for getting resistant plants than crossings with wild malus species detected by gene analysis. Information on the resistance of old local varieties against apple scab is insufficient because data were not gained under intensive orchard conditions.*

Keywords: Apple breeding, *Venturia inaequalis*, genetic diversity, old local varieties.

Introduction

The LVWO Weinsberg is doing research in organic apple production since 1987. One of the first topics of that work was the introduction of apple varieties which are resistant to *Venturia inaequalis* which is the main fungal disease in apple production. The genetic basis of all these new varieties was *Malus floribunda 821* (*Rvi6* resistance) which is still the main source of resistance of all commercially grown scab resistant apple varieties. In 2002 at the LVWO the breakdown of *Rvi6* (Vf) scab resistance was observed the first time in a range of apple cultivars grown in a research plot without any plant protection measurements, but also in an organic apple orchard nearby. In the following years the breakdown could be seen regularly every year in different stages of intensity. Since 2015 the LVWO is taking part in the VINQUEST-Project which is investigating the genes that are the best suited to breed apple cultivars with durable resistance (Bus et al. 2009, www.vinquest.ch). The aim of the project is to distribute host plants assigned to specific *Venturia inaequalis* races all across Europe to collect data in which parts of Europe which races of the fungus are established. At the moment there are 19 known differential hosts according to 19 races of *Venturia inaequalis*. In the orchards of the LVWO Weinsberg the hosts 0 to 15 were planted and showed in the last years that half of all scab races are present. Only host 5 (descendant of *Malus micromalus*), host 11 (descendant of *Hansen's baccata #2*) and host 15 (*Vr2*) did not show scab or signs of chlorosis and had a good vitality of the leaves.

Meanwhile, many of these resistance genes are well known and genetic markers are developed to detect them during the breeding process (Gardiner et al. 2007). In conflict to efforts of breeders to concentrate more than one source of resistance in new apple varieties is the fact that they used only few varieties as donor for fruit quality (Bannier, 2011). In 80 years of apple breeding mostly the varieties 'Golden Delicious', 'Cox Orange', 'Jonathan', 'McIntosh' and 'Red Delicious' were used as parents. Their descendants had a good fruit quality but also a loss of vitality. Meanwhile, in Germany and many other European countries a lot of collections of old local varieties exist ("landscape formative old varieties"). They are

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supposed to be tolerant to apple scab and mostly have a good vitality of leaves and trees. We speculate that the use of these old local varieties may be a solution to get a more sustainable resistance against *Venturia inaequalis* and more vitality in trees. Therefore, in 2016 a European Innovation Project (EIP) was started to investigate the fitness of old local varieties for the purpose of breeding.

Material and Methods

Crosses were performed in spring 2015 and 2016 with the aim to pyramid different scab resistances (*Rvi6, Rvi5, Rvi12, Rvi 15*, and from old local varieties). Parents were selected by gene analysis. As mother plants 'Flavia' (2015) and 'Natyra' (2016) were chosen which are both carrying the *Rvi6* gene and have a good fruit quality. Pollen was taken from the host plants of the VINQUEST project (*Rvi5, Rvi12, Rvi15*) and from 'Summercrisp' which is carrying the *Rvi10* gene (detected by gene analysis). The selection of parents from old local varieties was based on references from literature (Hartmann 2015, Bannier 2011). Seeds were extracted in autumn and subsequently stratified for 12 weeks in humid potting soil at 2-4°C to allow for regular germination in seed pots at the end of January. Seedlings were grown in the glasshouse at 18 to 20°C and artificially inoculated at the 6 to 8 leaf stage using a suspension of conidia dispersed in water at a concentration of 40,000 – 80,000 conidia per ml and high relative humidity for 48 hours. Inoculum was collected from scabbed leaves originating from apple trees not treated with fungicides. Infected leaves were taken in June from several locations and varieties (research fields, commercial orchards and from old local varieties) to represent the whole range of *Venturia inaequalis* races existing in the Neckar valley region. They were stored at -20°C. Three weeks after inoculation selection was done. Only trees without any symptoms of apple scab especially sporulating lesions were taken for further investigations. Molecular analysis was performed in collaboration with the company ecogenics GmbH, Balgach, Switzerland (www.ecogenics.ch). To select old local varieties for breeding purposes an orchard with 96 traditional apple varieties was excluded from fungicide treatments or another pest control in 2016 and 2017. Every variety was represented by two trees. They were screened for apple scab, powdery mildew, and susceptibility to *Marssonina coronaria*. Symptoms of the pathogens were evaluated on leaves and fruits using a 1-9 scale. Furthermore, apples were tested for their taste and storability in cold storage at 1-2 °Celsius. The cultivars were also examined for their organoleptic values.

Results

Inoculation with the mixture of the *V. inaequalis* races existing in the Neckar valley was very successful. In 2016 only 171 plants from 1,357 seedlings survived the selection procedure (12.6%). In 2017 from 478 plants 118 trees (24.7%) remained for further investigations. Reasons for the big loss of plants have to be seen in the strict selection but also in the presence of many scab races that have the potential to break a weak resistance. Pure *Rvi6* descendants have only little chance to survive the procedure, because the resistance has broken down (Table 2: crossing 'Natyra' x ('GoldRush' x 'Topaz')). Instead of an expected ratio of 50% survivors only 3.5% of the seedlings remained. Combinations of *Rvi6* with other resistance genes coming from host plants of the VINQUEST project were very successful. Up to 54% of the descendants survived the selection procedure. The following gene analysis showed that not all of them have a combined resistance but at least one resistance gene is present.

Table 1: Segregation of progenies with different scab resistance sources after the glasshouse seedling test in 2016 and number of plants with combined resistance.

Parents Mother Flavia Rvi6	Resistance genes	Plants before infection	Plants without symptoms	Percentage survivors	Plants with combined resistance
x Host 5	Rvi6 x Rvi5	210	65	30.9	23
x Host 12	Rvi6 x Rvi12	69	26	37.7	2
x Host 15	Rvi6 x Rvi15	85	35	41.2	11
x Prinz Albrecht	Rvi6 x ?	447	3	0.7	?
x Kardinal Bea	Rvi6 x ?	131	10	7.6	?
x C. Renette	Rvi6 x ?	343	18	5.2	?
x Sonnenwirt	Rvi6 x ?	72	14	19.4	?

Descendants of *Rvi10* (Summercrisp) showed a lower ratio of surviving plants (13.9%). Perhaps the reason for that is that not only one gene is responsible for the resistance but rather at least four (Bus et al. 2012). Not all of them will be found in a descendant at the same time. Descendants of old local varieties had the worst ratio of resistant or tolerant plants. Only 0.7% to 50% of the trees survived the selection procedure. The variety ‘Sonnenwirtsapfel’ brought the best success in both years. ‘Kardinal Bea’ and ‘Champagner Renette’ inherited low tolerance. Despite there were so many seedlings only a few trees stayed for further investigations. ‘Prinz Albrecht’ who was described in literature as very resistant to apple scab disappointed the most. From 447 seedlings only 3 did not get apple scab.

Table 2: Segregation of progenies with different scab resistance sources after the glasshouse seedling test in 2017 and number of plants with combined resistance.

Parents Mother Natyra Rvi6	Resistance genes	Plants before infection	Plants without symptoms	Percentage survivors	Plants with combined resistance
x (GoldRush x Topaz)	Rvi6 x Rvi6	114	4	3.5	-
x Host 5	Rvi6 x Rvi5	109	59	54.1	29
x Summercrisp	Rvi6 x Rvi10	187	26	13.9	8
x Seestermüher Zitronenapfel	Rvi6 x ?	8	3	37.5	?
x Dülmener Rosenapfel	Rvi6 x Rvi14	10	1	10.0	?
x Sonnenwirt	Rvi6 x ?	50	25	50.0	?

These investigations have been made in the preliminary stage of a European Innovation Project (EIP) to find suitable varieties for breeding out of the genetic pool of old local varieties. Despite the information out from literature the variety ‘Prinz Albrecht’ showed apple scab already from the beginning of the project when it was grown without plant protection. The varieties ‘Seestermüher Zitronenapfel’ and ‘Dülmener Rosenapfel’ seem to be interesting for breeding but there were too few seedlings to confirm that thesis. The selection of 96 old local varieties out of an orchard with no plant protection treatments for two years

resulted in some interesting varieties for breeding. ‘Kardinal Bea’, ‘Grahams Jubiläumsapfel’, ‘Gewürzluiken’, ‘Champagner Renette’, ‘Zuccalmaglio-Renette’ and ‘Sonnenwirtsapfel’ did not show any symptoms of apple scab in both years despite other varieties nearby were heavy infected. ‘Prinz Albrecht’ which was described as tolerant in literature was infected from the beginning of the experiment. Two years of organoleptic tastings of old local varieties in comparison to commercial cultivars showed that they were not competitive in taste.

Discussion

Since the collapse of the *Rvi6* scab resistance based on a single dominant gene from *Malus floribunda 821* breeders intend to stabilize the apple scab resistance by other genetic sources. The VINQUEST project is one effort in the direction to screen the pathotypes of *Venturia inaequalis* (Patocchi & Bus 2016). Up to now several genes have been found and can be detected by gene analysis. Old local varieties can be other sources for scab resistance. They have genes for partial resistance which can be pyramided into modern cultivars (Hampson 2013). But a high level of field resistance to scab is a poor predictor of a given parent’s breeding value. Crossings with old local varieties based on partial resistance need to have much more descendants for getting resistant plants than crossings with resistant varieties based on genes from wild malus species. Large populations will be necessary to find plants combining high scab resistance with good fruit quality. The artificially inoculation in the glasshouse with a mixture of conidia from the most important races of *Venturia inaequalis* is one method to reduce the amount of trees planted in the field. In the first stage of the breeding process the old local varieties have to be selected for suitable parents. Information from literature will be insufficient because the data were not gained under intensive orchard conditions. Further investigations for the selection of old local varieties under high density planting conditions without plant protection treatments have to be done.

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