Approaches in breeding high quality apples with durable disease resistance

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Abstract

*In organic apple production there is a rising demand for high quality and regularly bearing apple varieties with durable disease resistance. Relying only on single gene resistances such as Rvi6 (Vf) proved to be not a sustainable strategy as resistance breakdown is more and more frequently observed in orchards. Approaches followed by Agroscope in Wädenswil to pyramide several scab (*Venturia inaequalis*) resistances and combine them with powdery mildew (*Podosphaera leucotricha*) resistance and fire blight (*Erwinia amylovora*) tolerance are promising. In a joint effort, the EU project 'Fruitbreedomics' contributed to increasing efficiency in marker assisted breeding, a highly useful approach towards successfully pyramiding of resistances. The contribution of old local varieties to broaden the genetic basis in breeding, will also be highlighted.*

Keywords: apple breeding, *Venturia inaequalis, Podosphaera leucotricha, Erwinina amylovora,* genetic resources

Introduction

Breakdown of the *Rvi6 (Vf)* scab resistance in a range of apple cultivars especially in organic apple orchards by new pathogen strains virulent to *Rvi6* has become a serious problem in some apple growing areas of Europe. Despite breeding efforts during the last decades, currently the huge majority of commercially grown scab resistant apple varieties still carry the *Rvi6* resistance (Crosby *et al*., 1992; Laurens, 1999). Evidence in orchards shows that there are differences among varieties carrying *Rvi6* scab resistance: some are more vulnerable to resistance breakdown than others. From a genetic point of view, it would be challenging to elucidate the background of this phenomenon commonly attributed to unknown apple scab resistance QTL's (quantitative trait loci) or resistance gene effects masked by the *Rvi6* gene. In the scab resistance screening usually performed on young apple seedling at the 4 leaf stage in the glasshouse, different reaction classes: 0, 1, 2, 3a, 3b and 4 (according the Chevalier *et al*., 1991) are observed. This might reflect the presence of those additional genes underpinning the *Rvi6* resistance. Symptoms ranging from class 0 to class 3b are considered reflecting a resistance reaction. However, lower reaction classes, especially class 2, may be related to a higher and presumably more durable resistance than the higher ones such as 3a and 3b. Efforts to pyramide (combine) more than one scab resistance gene in a genotype are underway in breeding programmes and marketable products are in the pipeline (Baumgartner *et al.,* 2015). To date, more than 18 race-specific apple scab resistance genes have been identified and mostly mapped on different chromosome regions of the apple genome (Bus *et al.,* 2011, Jha *et al.,* 2009, Soriano *et al.,* 2009; Cova *et al*., 2015). Within the EU project 'Fruitbreedomics', a proof of concept of the pyramiding strategy was undertaken by inoculating progeny plants with different resistance gene setups with an apple scab strain overcoming the *Rvi6* resistance. The effect of the other resistance gene, (*Rvi2*), if present, became clearly obvious.

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The approach to breed new apple cultivars with pyramided scab resistances seems therefore promising by applying marker assisted breeding methods to efficiently detect plants with pyramided resistance. Simultaneously, powdery mildew resistance and fire blight tolerance are considered in the Agroscope breeding program. An alternative and complementary approach is to exploit the broad genetic basis in disease tolerance present in selected traditional varieties (Kellerhals *et al*., 2012; Gassmann *et al*.; 2014, Bastiaanse *et al*., 2015).

Material and Methods

Scab resistance pyramids

Apple progenies with scab resistance pyramids were developed by firstly characterizing potential parents by means of molecular markers closely linked to the relevant resistance genes. The pedigree of those potential parents were carefully analysed in order to verify that the expected resistance gene can be traced back to founders. Promising alternatives and/or complements for the *Rvi6* scab resistance constitute Rvi2 (*Vh2*), *Rvi4 (Vh4)* and *Rvi15* (*Vr2*) scab resistance. Moreover, advanced selections carrying *Rvi5* (*Vm*), *Rvi11* (*Vbj*) and *Rvi12* (*Vb*) were further developed towards cultivars with higher fruit quality. Basically, for all currently known major scab and powdery mildew resistance genes, reliable molecular makers are available. These are mainly SSR and a few SCAR markers. However, currently there is a shift towards applying SNP markers. SNP markers associated to apple scab, powdery mildew, rosy apple aphid (RAA) and fire blight resistance loci as well as some fruit quality related markers were made accessible for breeders also thanks to the European project 'Fruitbreedomics' and the US Project 'RosBreed' (Padmarasu *et al*., 2014; Jänsch *et al*., 2015; Cova *et al.,* 2015; Guan *et al.,* 2015) and applied in breeding progenies.

To evaluate the effect of the *Rvi2* gene in apple scab resistance pyramids, the *Rvi6* virulent strain EU-D42 (Caffier *et al*., 2015) kindly provided by R. Groenwold (Wageningen University and Research, NL) and V. Bus (Plant and Food Research, NZ) was applied in the quarantine glasshouse at Agroscope. Progeny plants from the two progenies (ACW 11303 (*Rvi6*) x ACW 18522 (*Rvi6, Rvi2*)) and (ACW 13652 (*Rvi6, Pl2*) x ACW 11567 (*Rvi2*)) were inoculated with scab (around 2 x 10^5 conidia per ml). Prior analyses with SNP markers allowed to build groups of plants with either no apple scab resistance gene, either Rvi6 or Rvi2, or Rvi6 and Rvi2 (=pyramid). Plants were evaluated four weeks after inoculation using the scoring scale of Chevalier *et al.* (1991) with additional classes for stellate chlorosis and stellate necrosis.

As for *Rvi12*, originating from *Malus baccata* 'Hansen's baccata #2' the resistance was dissected into two different resistance genes *Rvi12* and *Rvi12sc* (Patocchi, unpublished). Within the EU project 'Fruitbreedomics' a series of pre-breeding material was developed to broaden the genetic basis of scab resistance. Available donors are used in crosses with high quality parents.

Evaluation of traditional accessions towards their breeding value

At Agroscope, in the frame of the projects 'Description of fruit genetic resources' (03-NAP-P21 and 04-NAP-P21, BEVOG and BEVOG II), processed for the association 'Fructus', 600 traditional apple accessions were phenotypically screened from 2008 to 2015 for scab and powdery mildew incidence in an orchard not sprayed with any fungicide according to a scale 0 to 9 (0= no observation, 1= no visible symptom, 9= tree completely affected, nearly all the organs are infected) adapted from Lateur and Populer (1994). *Marssonina coronaria* severity was assessed with a similar scale in 2014 and 2015.

The evaluation of susceptibility and resistance to the most relevant plant diseases is an important aspect of those projects to describe and characterize the fruit genetic diversity preserved in Swiss genebanks (Kellerhals *et al*. 2012, Gassmann *et al*., 2014). Every accession was represented by two trees in a random design. Trees were grafted on M9T337 rootstock and spaced 3.5 x 1.5 m apart (1st leaf 2008). The most promising accessions were also tested for fire blight susceptibility in the glasshouse test with artificial shoot inoculation according to Baumgartner *et al*., 2015. Plant material preparation and inoculation, in a cabin of the quarantine glasshouse at Agroscope in Wädenswil, was conducted as described by Khan *et al*. (2006). Five to 12 replicate trees of each selection, showing active shoot growth and a minimal shoot length of 10 cm, were punctured with a syringe at the shoot tip with an *E. amylovora* solution (Swiss strain FAW610; 10^9 cfu/ml). Cultivar 'Gala Galaxy' was included as a susceptible control, and 'Enterprise' as a resistant control. The length of necrotic lesion (cm) was measured 7, 14, and 21 DAI (days post-inoculation). Shoot length was measured 7 DAI. The susceptibility of the selections and control cultivars was expressed in percentage lesion length (PLL) of the total shoot length and afterwards displayed as percentage relative to the susceptible cultivar 'Gala Galaxy' (= 100 %).

Results and Discussion

Scab resistance pyramids

A series of advanced selections with pyramided major resistances (Table 1 and http://www.fruitbreedomics.com/index.php/media-center/learning-material-training-sessions/154-prebreeding-material-and-germplasm) was developed. They are currently under further agronomic and pomological evaluation. These resistance pyramids are used as parents in breeding. However, while developing cultivars with such highly desired pyramided resistances, we have observed several genotypes showing fruit quality deficiencies. We anticipate that this may be due to linkage drag from the wild apple genome of the original resistance donors. Molecular markers could be used to identify seedlings showing a recombination close to the R-gene and a lower amount of genome originating from the wild apple ancestor.

Table 1: Examples of Agroscope selections carrying pyramided resistances against apple scab (*Rvi2, Rvi4, Rvi6*) and powdery mildew (*Pl1, Pl2*).

Selection Nb	**Traits**
ACW 16102	Rvi2, Rvi4, Rvi6, Pl1
ACW 16956	Rvi2, Rvi6, Pl2
ACW 18522	Rvi2, Rvi6

The analysis of the effect of the *Rvi2* gene in the two apple progenies segregating for scab resistance pyramids and inoculated with the *Rvi6* virulent strain EU-D42, gave encouraging results. In both progenies all plants with the genetic setup of *Rvi2Rvi6* (pyramid) and *Rvi2* alone were resistant (classes 0-3b according to Chevalier *et al*., 1991).

The mapping of the second apple scab resistance gene of 'Hansens baccata #2', temporary named *Rvi12sc*, and the subsequent availability of molecular markers associated to *Rvi12* and *Rvi12sc* allowed starting the re-evaluation of those genotypes that may carry both apple scab resistance genes of 'Hansens baccata #2'.

Pyramiding of disease resistance genes and loci should increase durability of disease resistant cultivars and therefore help fruit growers to establish more sustainable production. As for organic orchards, such cultivars should prevent growers to switch back, due to the spreading breakdown of the *Rvi6* scab resistance, to current commercially successful but disease susceptible varieties.

Evaluation of traditional accessions towards their breeding value

Among a set of 600 traditional apple accessions conserved in Swiss germplasm field repositories, 100 elite accessions in respect to low scab and mildew susceptibility were identified. Among those, based on a fire blight susceptibility evaluation with a shoot infection test in the glasshouse (Figure 1), as well as a preliminary fruit quality and storage disease rating, six elite accessions were identified (Table 2). The elite accessions will be used as breeding parents and should allow for broadening the still rather narrow genetic basis of the current commercial apple cultivars.

The data established during screening and evaluation of apple accessions in the framework of the "Swiss National Action Plan for the conservation of Plant Genetic Resources for Food and Agriculture" (NAP-PGREL) are displayed in the national data base for plant genetic resources (www.bdn.ch).

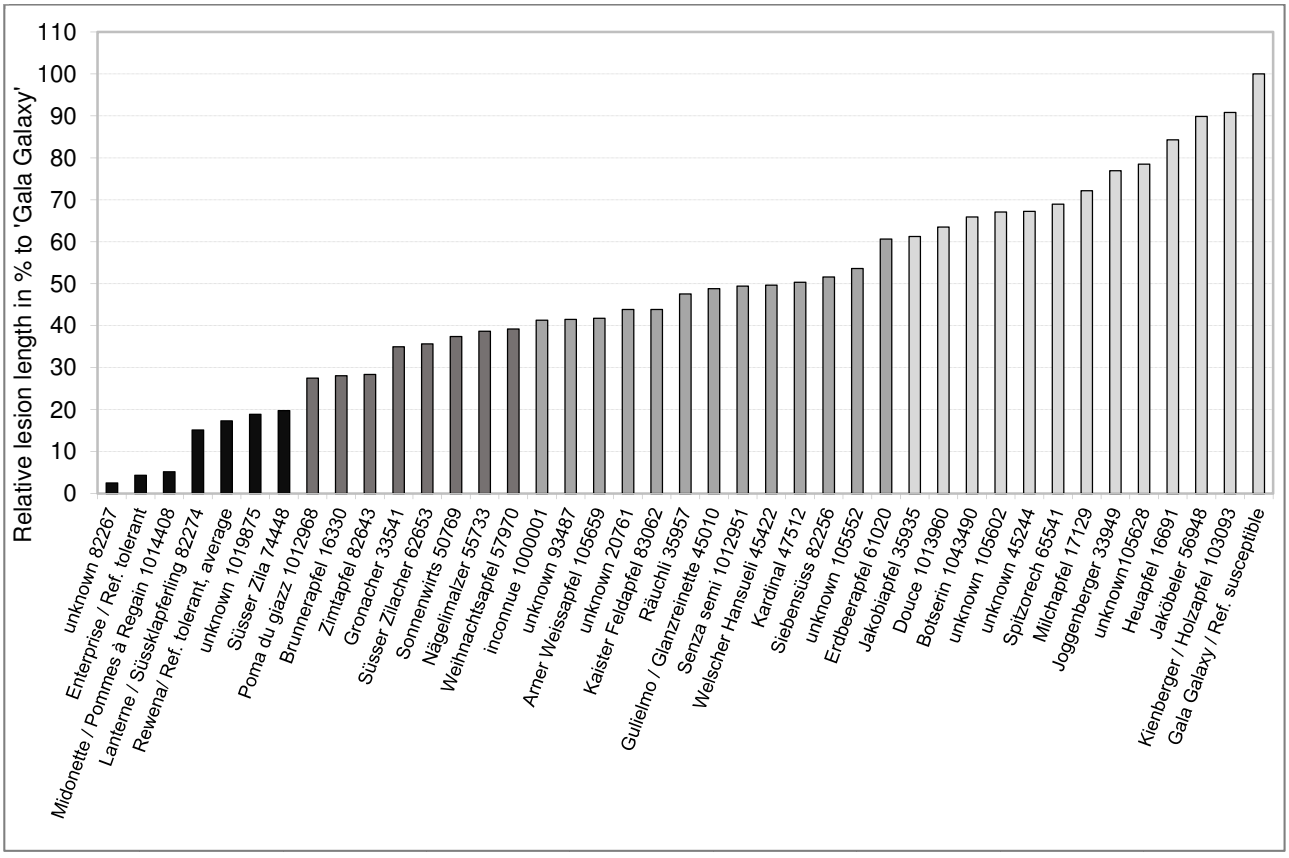

Figure 1: Mean lesion length 21 DAI in % to 'Gala Galaxy' of Swiss apple genetic resources accessions tested with the glasshouse shoot inoculation test. Cvrs. 'Enterprise' and 'Gala Galaxy' as tolerant and susceptible control, respectively.

Regarding the potential inherent to the broad diversity of apple genetic resources, there are promising initial steps for a successful use in breeding programs. Further in depth evaluation of accessions displaying an outstanding overall performance in respect to disease tolerance, fruit quality and production related features would be much appreciated by breeders.

Table 2: Evaluation results of outstanding accessions from a field trial with 600 traditional apple accessions

PL-Code	Accession name	Fire blight (% rel. to Gala Galaxy)	Scab (leaf scab) 1-9	Powdery mildew 1-9	Marsso-nina 1-9	Fruit quality score (1-9)
14-001-1491	Brunnerapfel	28.1	1	3	2	7
14-001-1514	unknown	43.9	2	3	3	6
14-001-1644	Sonnenwirts	37.4	3	2	3	6
14-001-1897	Kaister Feldapfel	43.9	1	4	2	7
14-001-2195	Senza semi	49.4	1	3	3	7
14-001-2226	unknown	18.9	2	2	4	6

An additional set of 750 apple accessions belonging to the Swiss inventory of fruit genetic resources will be evaluated for scab, powdery mildew and selected genotypes also for fire blight susceptibility in the coming years. Moreover, field scoring of *Marssonina coronaria* leaf blotch will be performed and as well as an evaluation of outstanding genotypes for fruit quality parameters and storage disease tolerance. The detected elite accessions will be used as breeding parents and should allow for broadening the still rather narrow genetic basis of the current commercial apple cultivars.

There is scope that in the near future apple varieties will be available either with a broader genetic disease resistance basis and/or with pyramided resistances against one single pathogen, such as apple scab, combined with other resistances and high fruit quality.

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