

## Modern approaches for breeding high quality apples with durable resistance to scab, powdery mildew and fire blight

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### Abstract

New methods to allow for more precise selection of tree and fruit characters in breeding programmes were developed in recent years. Marker-assisted selection (MAS) is common practice in the ACW apple breeding programme at Wädenswil. Genetic markers can reduce the number of plants and the time required for evaluation, thus new varieties become commercially available sooner. How can this molecular selection method reasonably be applied in an apple breeding programme? Application of phenotypic and molecular selection techniques in the ACW apple breeding programme and results are presented.

**Keywords:** apple, breeding, molecular markers, fire blight, scab

### Introduction

Apple breeding is a long-term and labour-intensive approach. Agroscope Changins-Wädenswil is carrying out an apple breeding programme for many decades and developed varieties such as Maigold, Arlet, Iduna, Ariwa (scab and powdery mildew resistant), Milwa (Junami®), La Flamboyante (Mairac®) and Galmac. The current breeding objectives include high fruit quality, good agronomic performance and durable disease resistance towards apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), and fire blight (*Erwinia amylovora*). Combining plant genomics and classical breeding is a challenge for breeders and molecular biologists. Several EU-projects such as EAGMAP (European Apple Genome Mapping Project), DARE (Durable Apple Resistance in Europe) and HIDRAS (High Quality Disease Resistant Apples for a Sustainable agriculture, QLK5-CT-2002-01-01492) have significantly contributed to progress in molecular apple breeding (King, 1996, Lespinasse et al., 2000, Gianfranceschi and Soglio, 2004). Marker-assisted selection (MAS) allows to speed up and facilitate the selection of novel cultivars. Some traits are determined by major genes and others by the additive effect of several genes (QTL's, Quantitative Trait Loci). For traits controlled primarily by single major genes, the Genome Scanning Approach has proven to be efficient to identify linked molecular markers, mainly SSR's (Patocchi et al., 2005). For traits controlled by several genes, usually the QTL mapping approach is applied. Additionally, van de Weg (2004) suggested an approach called Pedigree Genotyping. In contrast to the QTL mapping this approach is more flexible by not focussing on one single progeny but rather on small genetically related progenies, selections and varieties. To achieve durable disease resistance several functionally different resistances against the same pathogen can be combined. This approach can be followed thanks to molecular markers. Markers are available which are linked to the Vf, Vh2, Vh4, Vbj and other apple scab resistance genes (reviewed in Gessler et al. 2006) and the Pl1, Pl2, Pld and Plw mildew resistance (Markussen et al. 1995; Seglias and Gessler 1997; James and Evans 2004). To breed for fire blight resistant apple cultivars, genetic variation in the breeding material and in the Swiss apple germplasm collections is exploited. QTL's conferring fire blight resistance have been identified by Calenge et al. (2005) and Khan et al. (2006) in the cultivar 'Fiesta'.

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For this QTL Khan et al. (2006) developed two markers AE 10-375 and GE-8019 one on each side of the QTL. For fire blight no major resistance genes have yet been found. However, Peil et al., 2007 assumed a major resistance gene for fire blight on linkage group 3 of the crab apple *Malus x robusta* 5. We mainly rely on cultivars and selections with known low susceptibility.

Breeding strategies are continuously adapted to new methods and technologies and include classical and molecular selection techniques.

### Material and Methods

The breeding scheme applied by ACW is shown in Fig. 1. The figure displays the way of the annually produced 10'000 seedlings to two outstanding selections that are candidates to become a new variety. On average one commercial variety out of about 30'000 original seedlings can be expected. The most advanced and promising breeding material and commercial varieties but also old varieties or wild species with specific characteristics are used as parents. The selected parents are subjected to molecular analysis to confirm the presence of desired genes.

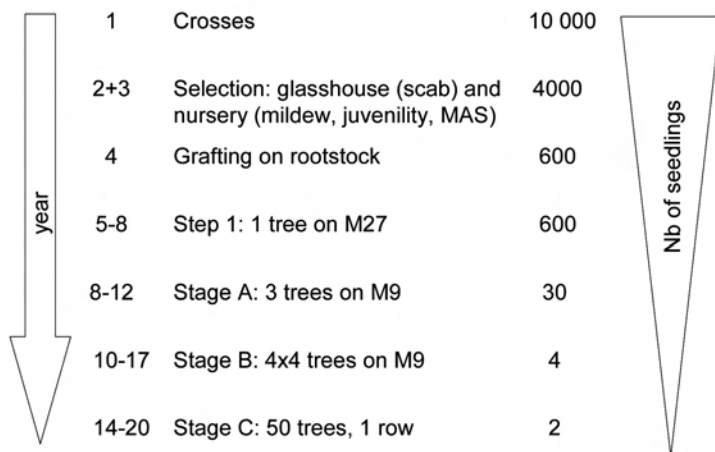


Fig. 1: Apple selection scheme at ACW Wädenswil

The first selection step is the glasshouse screening for scab resistance. When major resistances such as *Vf*, *Vh2*, *Vbj*, etc. are incorporated, plants are inoculated with a solution of 350'000 conidia/ml and subsequently after 2 weeks scored according to Chevalier et al., 1991. While dealing with partial (polygenic) scab resistance, a ten times lower spore dosage is applied and the scale of Lefrancq. et al., 2004 used. Seedlings scored as being resistant (Classes 0 to 3b on the scale of Chevalier et al., 1991 and 0 to 3 on the Lefrancq et al., 2004 scale) are planted to an outdoor nursery field. In the second year they are selected for mildew resistance, vigour and absence of juvenile symptoms. At this stage MAS is applied to progenies where resistance genes have been pyramided to identify plants carrying two resistance genes. DNA extraction is performed according to Frey et al. (2004). Subsequently using the liquid handling robot epMotion 5075 (Eppendorf), multiplex PCR's with fluorescently labelled primers are assembled. Fragment analysis is carried out on a 3130xl Genetic Analyzer (Applied Biosystems) and data analysis is done with GeneMapper™ Software (Applied Biosystems).

By means of the phenotypic glasshouse selection for scab resistance, MAS and the selection in the nursery field, the total number of seedlings is reduced from originally about 10'000 to 600 at the age of 2 years. Those selected seedlings are grafted on the dwarfing rootstock M 27 to evaluate tree and fruit performance.

The screening of advanced selections and potential parents for fire blight tolerance was conducted in the ACW quarantine glasshouse. Scion material was grafted onto M9 rootstock. In spring 2007, trees were planted in plastic deep-pots 60 from Stuewe & Sons (Corvallis, US) with a length of 35.5 cm and diameter of 7 cm and then grown in the glasshouse for several weeks prior to inoculation. For each variety, 10 replicate trees were inoculated by puncturing the distal tip of shoots 15-30 cm long with a syringe containing an *E. amylovora* solution of 10<sup>6</sup> cfu/ml strain CFBP1430 (INRA, France). Spreading of disease symptoms was evaluated in weekly intervals during three weeks by measuring the expansion of the necrotic lesion from the shoot tip in relation to the total shoot length.

Fruit quality is evaluated by sensory analysis from step 1 to stage C testing (see Fig.1) with increasing precision towards higher stages. Whereas in step 1 usually only one person is evaluating the genotypes, several evaluators are included and additional instrumental analyses are performed during stage A. Gradually expert panels and finally consumers are included (Kellerhals and Eigenmann, 2006).

## Results and Discussion

MAS is currently applied mainly for specific and pre-selected seedling progenies in their second leaf. The progeny Topaz x FAW 11567 is an example. The cross was made in 2004. FAW 11567 (Milwa x Reka) is a parent with good fruit quality and carrying *Vh2* scab resistance originating from the crab apple Russian seedling R12740-7A and Topaz carries the *Vf* resistance, which was confirmed by marker analysis. The population comprised 2076 seedlings. Phenotypic pre-selection was performed in the glasshouse using the scale of Chevalier et al., 1991. 1679 seedlings were classified as resistant (80.9 %). Out of these seedlings, 462 were phenotypically selected in the second year in the nursery field and included in a molecular analysis using the CHVf1 marker for *Vf* and the markers CH02b10 and CH05e03 flanking *Vh2*. The marker analysis revealed 134 seedling that carried all three markers and therefore the two scab resistance genes pyramided.

Table 1 presents advances achieved at ACW in breeding selections with pyramided scab resistance combined with mildew resistance. Marker analysis allows to verify expected genetic constitution. For hybrids 16102 and 16208 molecular analysis has shown that instead of the two expected scab resistance genes, 3 resistance genes against scab were present: *Vf*, *Vh2* and *Vh4*. According to Bus et al., 2005, the Russian apple R 12740-7A carries three major resistance genes: *Vh2*, *Vh4* and *Vr*. Additionally the genotypes 16102 and 16208 carry PI1 and PI2 mildew resistance, respectively. However, selection 16254 did not carry the expected PI1 resistance. Fruit quality is reasonably good in these selections. Selection for tree and fruit characteristics will continue in this material in order to find the genotypes with the best overall performance.

Table 1: Molecular analysis of hybrids expected to carry pyramided resistance against scab with at least two different genes and a mildew resistance (+ = marker present, - = marker absent)

Hybrid	Cross	Genes expected	Marker for Vf	Marker 1 for Vh2	Marker 2 for Vh2	Marker for Vh4	Marker for PI1	Marker for PI2	Fruit quality
16102	Ariwa x Regia	Vf, Vh4, PI1	+	+	+	+	+	-	good
16208	FAW 8259 x FAW 11561	Vf, Vh2, PI2	+	+	+	+	-	+	medium
16254	Ariwa x Reka	Vf, Vh2, PI1	+	+	+	-	-	-	good

First attempts to establish MAS for fire blight resistance combined with scab resistance gave encouraging results but also need further work. Table 2 shows the molecular analysis of a progeny FAW 9991 x Enterprise. Both parents carry the *Vf* scab resistance in a heterozygous situation. The entire progeny consisted of 509 seedlings of which 379 plants were phenotypically scab resistant (75.1%) which perfectly corresponds to a 3 resistant to 1 susceptible segregation of two parents heterozygous for *Vf*. Of these 379 plants, 266 are heterozygous and 104 are homozygous for *Vf*, therefore a 2:1 segregation as expected.

Table 2: Molecular analysis of the progeny FAW 9991 x Enterprise for markers related to fire blight resistance (AE 10-375 and GE-8019; + = marker present, - = marker absent)

AE10-375	GE-8019	Nb of Plants
+	+	<b>184</b>
+	-	93
-	+	4
-	-	89
+	-	FAW 9991
+	+	Enterprise

Enterprise carries both molecular markers associated to the 'Fiesta' 7 fire blight QTL while FAW 9991 carries only the marker AE 10-375. Plants amplifying both markers AE 10-375 and GE-8019 carry the QTL allele conferring increased resistance to fire blight. 184 scab resistant progeny plants (139 *Vf/vf* and 45 *Vf/Vf*) were identified with this marker combination. Plants carrying both markers for the fire blight resistance QTL and the *Vf* resistance at a homozygous level will be preferentially selected.

Glasshouse screening of advanced selections with a shoot inoculation test for fire blight resistance highlighted considerable differences among selections (Figure 2). Genotypes such as FAW 14995 and FAW 12309 displayed low susceptibility to fire blight. They are interesting as breeding parents and potentially as cultivars. However, further testing under field conditions is required to confirm their low susceptibility to fire blight. As concerns their potential interest as commercial cultivars a whole range of other selection criteria need to be fulfilled as well. Similar tests were performed with heritage varieties from genetic resources collections. Among 20 different apple accessions tested in 2007 a huge variation in respect to susceptibility and tolerance was found.

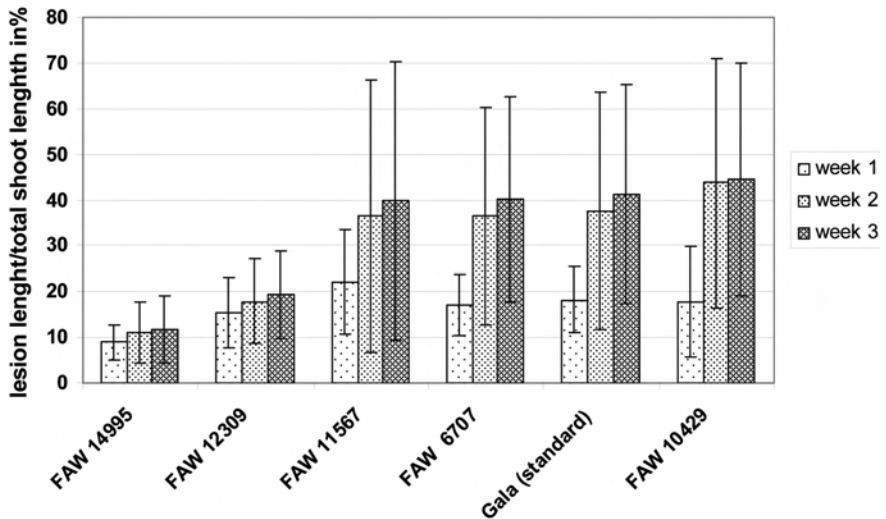


Fig. 2: Development of fire blight in the shoot infection glasshouse test 2007 at ACW Wädenswil (n=10 plants/genotype, bars represent standard deviation)

Fruit quality is the most important breeding objective. The success of newly developed disease resistant apple varieties is largely dependent on their fruit quality (Kellerhals and Eigenmann, 2006). Fruit quality includes many quantitative traits. First fruits can usually be expected at earliest in the fourth year after crossing. However, usually the first fruit selection step is carried out from the fifth to the seventh year after crossing. For advanced selections, the methods and tests gradually become more sophisticated. Expert panels, instrumental and sensory analysis at different stages of storage are performed and finally consumer tests should give answer as to how the market chances can be rated. Within the HIDRAS project research was carried out also at ACW to develop and apply marker-assisted selection for fruit quality traits such as flesh firmness and acidity. MAS for fruit quality traits would considerably increase selection efficiency, as fruit quality traits can only be selected relatively late in the selection process. However, the development of high throughput systems for MAS is also necessary in order to apply this method in an economically reasonable manner to large progenies.

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