Breeding apples with durable disease resistance

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Abstract

Apple cultivars with high fruit quality, good productivity and durable disease and pest resistance are highly required in ecological fruit growing. In the past apple breeders mainly used single resistance genes giving good field resistance towards scab and mildew. They originate from wild species such as *Malus floribunda* 821, *M. zumi* and *M. robusta*. However, this type of resistance can be overcome by new pathogen races. Today the main emphasis is on the combination of functional different major resistance genes and the use of quantitative resistance to achieve durability. Molecular markers were developed which particularly permit the detection of major resistance genes. The combined presence of the target resistance genes in a seedling can be determined by marker-assisted selection (MAS). Currently similar markers for loci on the genome which determine quantitative resistance (QTL) are being developed. Benefits and limits of durable disease resistant varieties in organic fruit growing will be discussed.

Keywords

Apple, breeding, *Venturia inaequalis, Podosphaera leucotricha, molecular markers*

Introduction

Apple breeding at Wädenswil is aimed at combining high fruit quality with good orchard performance and durable disease resistance for sustainable production systems. Ariwa is a commercially available scab *(Venturia inaequalis)* and mildew (*Podosphaera leucotricha*) resistant cultivar (Vf and PI_1) developed at Wädenswil. Further advanced selections (Table 1) are evaluated at FAW Wädenswil, under organic growing systems at FiBL Frick and in other trials.

Table 1: Variety and advanced selections developed by FAW Wädenswil

Variety, selection	Parentage	Resistances	Fruit
Ariwa	Golden Delicious x A 849-5	Vf, PI_1	Orangered, very firm, slightly sweet
FAW 7242	Gala x Florina	Vf	Dark red, firm, sweet
FAW 8244	Gala x E 34-120	Vf, PI_2	Red, firm, well-balanced flavour
FAW 11303	FAW 6104 (Arlet x Gloster) x Rewena	Vf, MR	Dark red, firm, crisp, juicy

Vf: scab resistance PI_1, PI_2, MR: mildew resistance

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The durable incorporation of scab and mildew resistance can reduce the input of fungicides considerably. The great majority of released scab resistant apple varieties carry the Vf scab resistance originating from *Malus floribunda* 821. However, this type of mainly monogenic resistance has shown to be subject to resistance breakdown under severe infection conditions (Parisi et al., 1993, Fischer 1998). Race 6 is virulent to most of the Vf resistant cultivars, but avirulent to the progenitor *Malus floribunda* 821 (Parisi and Lespinasse, 1996). Race 7 was found in England (Roberts and Crute, 1994). This race is virulent to *Malus floribunda* 821 but avirulent to Golden Delicious. The resistance of Golden Delicious to race 7 is due to a single gene called Vg (Bénaouf and Parisi, 1997). In Wädenswil race 7 was detected in 1999 on trees of *Malus floribunda* 821 and on trees of cv. Topaz not treated with fungicides (unpublished by the authors).
A current EU project, DARE (**d**urable **a**pple **r**esistance in **E**urope, Lespinasse et al., 2000, Kellerhals et al. 1999), aims to establish durable disease resistant varieties through pyramiding resistance genes and resistance types. In particular polygenic resistance and major resistance genes should be combined. Polygenic or partial resistance, based on a quantitative genetic support is considered to be durable (Parlevliet 1993). The relatively new tool of molecular markers allows breeders to select at an early stage for characters which are not in any case detectable phenotypically. Only a small amount of DNA is needed which at the limit could already be extracted from the seed tissue.
Progress in marker-assisted selection for apple breeding is being achieved mainly in the area of disease resistance. The development of QTL markers for polygenic resistances and for different major resistance genes will allow a broad application of marker-assisted selection. However, this largely depends on the economic feasibility of this approach: the costs for the marker analysis should be relatively low in order to allow screening of large populations.

Pyramiding resistances against scab

In breeding programmes markers can be used to select those progeny plants that have the best possible combination of desired characters. Marker assisted selection is supposed to be extremely useful in the process of pyramiding (combining) resistance genes against the same pathogen (table 2). It is possible to pyramide different major genes (qualitative traits) or to pyramide major genes and polygenes, more correctly called quantitative trait loci (QTL). Markers for scab and mildew QTL resistance are being developed in the course of the DARE project. The Swiss Federal Institute of Technology (ETH Zürich) has developed a range of useful markers for disease resistance studies in apple. Currently markers for the scab resistance genes Vbj and Vr are being developed and further improved for the use in marker-assisted selection.
MAS is applied at Wädenswil. The progeny of Ariwa x FAW 7630 was analysed in detail. Ariwa carries Vf resistance against scab and Pl_1 resistance against mildew. FAW 7630 was thought to carry Vbj scab resistance. However, analysis with microsatellites indicated that this is not the case. Leaves were detached from seedlings and DNA extracted according to Kellerhals *et al.* (2000). For detection

of Vf PCR was performed with AL07-SCAR marker (Tartarini *et al.*, 1999) and for Pl1 the AT20 marker was applied (Markussen *et al.*, 1995).

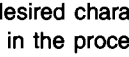
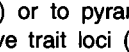
Table 2: Examples of crosses made in the year 2000 to pyramide scab resistances and to combine scab and mildew resistance

Mother	Father	Nb. of seeds	Remarks
Ariwa(Vf, Pl_1)	Reka (Vr)	1244	Combination Vf, Vr, Pl_1
FAW 8244 (Vf, Pl_2)	FAW 8476 (Vbj)	430	Combination Vf, Vbj, Pl_2
FAW 8159 (Vf, Pl_1)	FAW 12364 (Vdisco)	1021	Combination Vf, Vdisco, Pl_1
Goldrush (Vf)	FAW 8244 (Vf + Pl_2)	812	Vf homozygous, Pl_2

Vf, Vr, Vbj, Vdisco = genes for scab resistance Pl_1, Pl_2: genes for mildew resistance

Phenotypic scab screening is performed under glasshouse conditions. The seedlings are inoculated with local scab inoculum with a dose of 370'000 conidia per ml under high humidity and a temperature of ideally 18-20C. Scoring of the symptoms is done after 2 weeks according to the scale of Chevalier et al.(1991).

Table 3: Phenotypic scab reaction classes in the glasshouse screening according to Chevalier et al., 1991

Class	description	
0	resistant	No visible symptoms
1		pin-point pits (usually not present or only in few cases)
2		Chlorotic lesions, slight necrosis, no visible sporulation
3a		Necrotic lesions, some chlorotic lesions, occasionally slight sporulation
3b	moderately susceptible	Chlorotic and necrotic lesions, sporulation
4	susceptible	Abundant sporulation

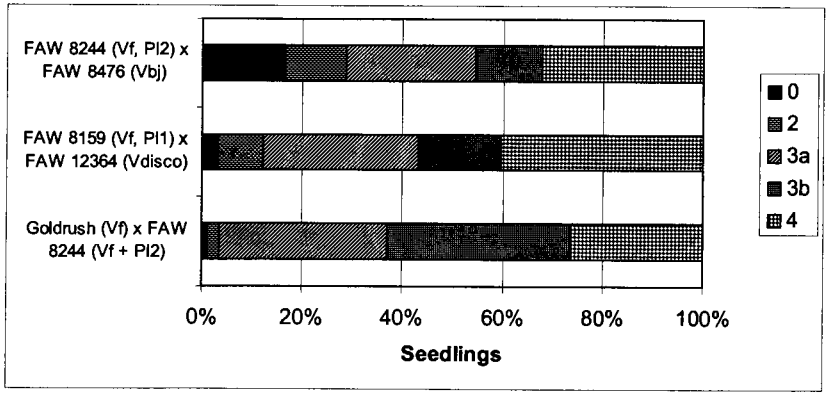


Figure 1: Phenotypic segregation of selected crosses 2000 in the glasshouse screening

A set of 378 seedlings was scored phenotypically and analysed for the presence or absence of AL07-marker for the Vf resistance. For marker analyses a young leaf is detached from each seedling for DNA extraction before scab inoculation. The correlation between phenotypic classification and presence or absence of the marker was very good (Table 4).

Table 4: Phenotypic and molecular segregation for Vf marker ALO7 in the cross Ariwa x FAW 7630 (378 seedlings)

	phenotypic	molecular
Susceptible	46.4 %	46.7%
Resistant	53.6 %	53.3%

In order to broaden the genetic basis of disease resistance, fruit quality and growth habit types, crosses were made with genetically distant parents carrying partial scab resistance and known to be moderately mildew susceptible (Figure 2). The polygenic VA scab resistance derived from 'Antonovka' showed good segregation in resistant and susceptible progeny. However, in the other combinations the share of resistant seedlings was rather low. This fact is well-known and a main reason for the abundant use of the Vf resistance which usually transmits a more or less 50:50 segregation between resistant and susceptible progeny.

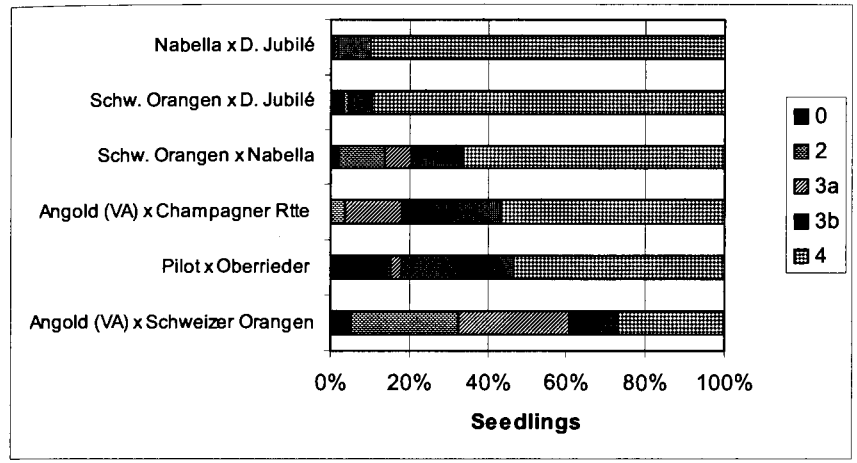


Figure 2: Phenotypic segregation in cross combinations with parents carrying partial scab resistance

MAS is still expensive and therefore suitable only for specific aspects such as gene pyramiding or early screening of traits which can usually only be evaluated at a late developmental stage. Currently we are looking for ways to reduce cost. A quick extraction method for apple DNA has already been developed (Dilworth and Frey, 2000).

Benefits and limits of disease resistant varieties

Traditional cross breeding combined with marker-assisted selection considerably contributes to enhance sustainable apple growing systems. It allows developing high quality apple cultivars with durable resistance. Progress is under way in respect to combining scab and mildew resistance and to enlarge the genetic background of the resistances. However, it will always be necessary to establish a resistance management to prevent resistance breakdown. Moreover, it does not seem possible to solve all disease and pest problems in the apple through breeding. Resistance targeted to fungi causing diseases such as sooty blotch and fly speck is probably not available in nature, and a more general resistance would most likely involve unwanted changes such as peel resistance. Combination with orchard management as well as with environmentally and consumer friendly plant protectants are necessary. For the time being, it is advised not to plant disease resistant varieties carrying single resistance genes such as Vf without any plant protection, nor to neighbour susceptible cultivars with resistant cultivars. Besides lowering the selection pressure on the pathogen population by the Vf resistance, such minimal control measures reduce mildew incidence, fire blight risk and additional problems such as sooty blotch, fly speck and other pathogenic agents with might be detrimental for tree and fruit quality.

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