

# Suitability of old apple varieties in organic farming, based on their resistance against apple scab and powdery mildew

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Received: 16 October 2014 / Accepted: 16 June 2015 / Published online: 30 June 2015  
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**Abstract** Apple scab and powdery mildew are the two most destructive fungal diseases of apple, especially in organic orchards, where synthetic pesticides are prohibited. Only a limited choice of natural substances for control of pests and diseases allowed to be used. Growing resistant/tolerant cultivars can highly reduce the number of the necessary plant protection treatments resulting in lower costs and environmental impact. Besides the newly developed cultivars, several old apple cultivars are thought to be resistant/tolerant to the main fungal diseases of apple; however, in most cases, the degree and type of their resistance is not well characterized. To evaluate the resistance of ten old cultivars against apple scab and powdery mildew, field evaluations were carried out for 6 years. Molecular marker analysis was also carried out: six different markers were used for the detection of three major scab resistance genes (*Vf*, *Vh4*, *Vh2*). The resistance gene *Vf* could not be found in any old cultivar while the resistance genes *Vh2*, *Vh4* are possibly present in several cultivars. The results are explaining the good disease tolerance of several cultivars (e.g., ‘Batul’), and also suggest their relevance in the breeding as well as in organic farming. Some old cultivars showed good field resistance against fungal diseases, even though their resistance against scab could not be explained with the presence of the

investigated major scab resistance genes. This suggests that a few cultivars might possess polygenic resistance, or maybe new major scab resistance genes.

**Keywords** *Venturia inaequalis* · *Podosphaera leucotricha* · Field evaluation · Marker analysis

## Introduction

The two most important fungal diseases of the domesticated apple (*Malus × domestica* Borkh.) are the apple scab (*Venturia inaequalis* Cke.) and powdery mildew (*Podosphaera leucotricha* Salm.) which are both responsible for huge economical loss worldwide in every year. If we apply integrated pest management, then the costs of the sprays against fungal diseases can exceed even the half of the full production costs. In addition, the pesticides permitted in organic farming have low efficiency compared to the chemicals usable in conventional growing which increases the importance of alternative defense strategies, such as the use of resistant cultivars (Holb et al. 2005; MacHardy 1996; Reuveni et al. 2000).

Cultivars which are at least tolerant to the major fungal diseases (apple scab and powdery mildew) are needed especially by organic farmers. Recognizing the increasing need of the market for resistant cultivars, resistance breeding program was started in the 1990s by the Department of Pomology, Corvinus University of Budapest, with the aim of producing new apple cultivars with excellent fruit quality and resistance against the major diseases of apple [apple scab, powdery mildew

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and fire blight (*Erwinia amylovora* Burrill, Winslow et al.)). As part of the program, many old apple cultivars of the Carpathian Basin were collected, and the collection of fruit cultivars located in Soroksár had been established. Besides their relevance in the breeding, many of these cultivars have potential in the organic farming as well, due to their good disease resistance, fruit quality, and suitability to less intensive technology (Tóth et al. 2005).

The method of molecular marker analysis gives us new opportunities to evaluate the resistance of the cultivars. Markers of resistance genes are often used to fasten the breeding process by tracking the inheritance of the R genes (marker assisted breeding). In the same time, they are also capable to strengthen the results of the phenotypic evaluations, which is necessary, because the development of disease symptoms are influenced by several factors (ecological conditions, pest control, composition of the present pathogen races, etc.). To make a stable statement about the degree of resistance of a cultivar, we have to understand the host-pathogen interactions, and clarify the genetic background of the resistance, as well (Kumar et al. 2011).

The polygenic or quantitative apple scab resistance is based on several genes, and its effect is very variable (from low to high degree of resistance). As it is not race specific, it is more stable than the monogenic resistance; however, it is very difficult to inherit it to a seedling. In contrast to this, the monogenic or qualitative resistance is based on one single gene so it can be inherited easily with crossing. Its effect is based on gene-for-gene interaction between the pathogen and the host, and typically characterized with hypersensitive response. Though the polygenic resistance has still potential in the breeding, as it is hard to inherit it, the breeding rather relies on the small fruited apple species (*Malus* sp.) containing major resistance genes (having monogenic resistance). The *Malus baccata* jackii (*Vbj*), *Malus baccata* (*Vb*), *Malus micromalus* and *Malus atrosanguinea* (*Vm*), the *Malus pumila* ‘R12740-7A’ (*Vr*), and the most significant *Malus floribunda* (*Vf*) species all played a role in the production of the modern scab resistant apple cultivars as gene sources (Gessler et al. 2006).

As the monogenic resistance is race specific, those pathogen strains which genome does not contain the proper avirulence factor can affect even the otherwise resistant plants. The composition of scab races thus the resistance of cultivars may vary in space and time depending on the environmental factors (Sierotzki et al. 1994). As a result of the discovery of new *V. inaequalis*

races which are able to infect the *Vf* resistant cultivars (which was thought to be the most stable major gene), the strategy of inheriting one single resistance gene in order to produce new resistant cultivars is no longer preferred by the breeders (Parisi et al. 1993). To achieve more stable resistance, and avoid the occurrence of new pathogen races, the modern approach is to accumulate the different resistance genes (possibly several major genes) in one cultivar, thus pyramiding resistance genes (Broggini et al. 2011; Soriano et al. 2009).

The identification of resistance genes in different genotypes and the identification of pathogen races in different regions are an intensely studied field. Also, the nomenclature of the major scab resistance genes has been recently updated according to the gene-for-gene interaction between the avirulence gene of the pathogen and the resistance gene of the host (Bus et al. 2009). Nevertheless, in this study, we stick to the old names of the genes for the better understanding.

Several SCAR and SSR markers linked to the major apple scab resistance genes have been developed and suggested to the breeders, and it has been concluded that the use of these markers is also necessary for the modern evaluation of cultivars (Patzak et al. 2011; Urbanovich and Kazlovskaya 2008; Patrascu et al. 2006; Patocchi et al. 2009).

In the present study, our aim was to evaluate the apple scab and powdery mildew resistance of ten old apple cultivars on the field (in the collection of fruit cultivars located in Soroksár), and carry out genetic marker analysis, to possibly locate apple scab resistance genes in the investigated genotypes. The new data from one side may help to select new resistance sources for the breeding, as well as it helps to characterize precisely the disease resistance/susceptibility of the cultivars which is one of the most important characteristic for organic farmers.

## Methods

### Evaluation of the scab and powdery mildew resistance on the field

The apple scab and powdery mildew resistance of the investigated old cultivars were evaluated on the field in the cultivar collection located in Soroksár (Hungary). The gene bank is under integrated pest

management; however, mild scab and mildew infection occurs year by year.

Four trees were studied per cultivar for 6 years, and observations were made at least two times per year. In the case of apple scab, we evaluated the symptoms on the leaves, while the infected shoot tips were observed in the case of powdery mildew. In both cases, the percentage of the infected leaves or shoots thus the incidence values of the pathogen were noted. Then we categorized our cultivars in all year using a common four-grade scale derived from the incidence values: S—susceptible, MS—moderately susceptible, MR—moderately resistant, and R—resistant.

### Molecular marker analysis

The plant material was collected in Soroksár, and stored under  $-20\text{ }^{\circ}\text{C}$  until processing. The DNA extraction from young leaf tissue or bud was made with QIAGEN DNeasy® Plant Mini kit (Hilden, Germany).

Four SCAR and two SSR markers were used in order to detect four major scab resistance genes. The properties of the used markers can be seen in Table 1. The AL07 and the dominant AM19 markers were used to detect the *Vf* gene, while the AD13 and OPL19 were used to detect the *Vh4* and *Vh2* genes, respectively. We used the SSR markers CH02c02a and CH05e03 to straighten our results concerning *Vh2* and *Vh4* genes.

PCR was performed with a final volume of 16  $\mu\text{l}$  containing Taq polymerase (Fermentas, Canada). The following conditions described by Boudichevskaia et al. (2006) were used to amplify the AD13, AL07, and AM19 SCAR markers: one cycle of 2 min  $94\text{ }^{\circ}\text{C}$ ; 30 cycles of 1 min at  $94\text{ }^{\circ}\text{C}$ , 3 min at  $58\text{ }^{\circ}\text{C}$ , and 2 min

at  $72\text{ }^{\circ}\text{C}$ ; and a final extension cycle of 10 min at  $72\text{ }^{\circ}\text{C}$ .

The amplification conditions for OPL19 were as described by Bus et al. (2005): one cycle of 2 min 45 s at  $94\text{ }^{\circ}\text{C}$ ; 40 cycles of 55 s at  $94\text{ }^{\circ}\text{C}$ , 55 s at  $55\text{ }^{\circ}\text{C}$ , and 1 min 39 s at  $72\text{ }^{\circ}\text{C}$ ; and a final extension cycle of 10 min at  $72\text{ }^{\circ}\text{C}$ . For the amplification of the SSR markers, we used the following conditions, described by Patocchi et al. (2009): one cycle of 15 min at  $94\text{ }^{\circ}\text{C}$ ; 40 cycles of 40 s on  $94\text{ }^{\circ}\text{C}$ , 1 min 30 s on  $55\text{ }^{\circ}\text{C}$ , and 1 min 30 s on  $72\text{ }^{\circ}\text{C}$ ; and finally 30 min at  $60\text{ }^{\circ}\text{C}$  and 10 min at  $72\text{ }^{\circ}\text{C}$ . The PCR products of the SCAR markers were separated on 2 % agarose gel, while the fragment analysis of the SSR markers was made by the Agricultural Biotechnology Center (Hungary) and was evaluated with the Peak Scanner software (Applied Biosystems).

## Results

### Field evaluations

In the period of 2010 to 2015 (in Soroksár, Hungary), the investigated cultivars showed very high disease resistance, while the susceptible reference cultivars—‘Gala’ for apple scab and ‘Jonathan’ for powdery mildew—had considerable symptoms. All of the investigated cultivars proved to be at least tolerant to scab in all years except 2013 (see Table 2). The ecological conditions were the most favorable for the disease in the year of 2013, thus both ‘Máté Dénes’ and ‘Vilmos renet’ showed moderate susceptibility; however, they showed no symptoms in the other years.

**Table 1** The properties of the used markers

Marker	Size (bp)	Type	R gene	Dominance	Reference
AL07	570	SCAR	<i>Vf</i>	Codominant	Tartarini et al. 1999
AM19	526	SCAR	<i>Vf</i>	Dominant	Tartarini et al. 1999
AD13	950	SCAR	<i>Vr1/Vh4</i>	Codominant	Boudichevskaia et al. 2006
OPL19	433	SCAR	<i>Vr1/Vh2</i>	Codominant	Bus et al. 2002
CH05e03	173	SSR	<i>Vh2</i>	Codominant	Bus et al. 2005
	165		<i>Vh4</i>		
CH02c02a	170	SSR	<i>Vr1/Vx1/Vh4</i>	Codominant	Bus et al. 2005

**Table 2** Scab resistance on the field of the ten selected old apple cultivars

Cultivar	2010	2011	2012	2013	2014	2015
Gala <sup>a</sup>	S	S	S	S	S	S
Batul <sup>b</sup>	R	R	R	R	R	R
Citrom alma	R	R	R	R	R	R
Kanadai renet	R	R	R	R	R	R
Máté Dénes	R	R	R	MS	R	R
Pónyik alma <sup>b</sup>	R	R	R	R	R	R
Sándor cár	R	R	R	R	R	R
Sikulai <sup>b</sup>	R	R	R	R	R	R
Szabadkai szercsika <sup>b</sup>	MR	R	R	R	R	R
Tordai piros kálvil <sup>b</sup>	R	R	MR	S	R	R
Vilmos renet <sup>b</sup>	R	R	R	MS	R	R

*R* resistant, *MR* moderately resistant, *MS* moderately susceptible, *S* susceptible

<sup>a</sup>Susceptible control

<sup>b</sup>Cultivars previously recommended by Tóth et al. (2005)

The degree of powdery mildew resistance on the field is shown in Table 3. The years 2012 and 2015 could not be evaluated, since even the highly susceptible control cultivar ‘Jonathan’ had no or only had moderate symptoms. The ‘Tordai piros kálvil’ showed considerable amount of symptoms in the year of 2011, while heavy infection occurred in 2014, resulting in moderate symptoms on ‘Kanadai

renet’ and ‘Szabadkai szercsika’ All the other cultivars were uninfected in the period of the study.

#### Marker analysis

The results of the marker analysis carried out to detect the major scab resistance genes are shown in Table 4.

**Table 3** Powdery mildew resistance on the field of the ten selected old apple cultivars

	2010	2011	2012	2013	2014	2015
Jonathan <sup>a</sup>	S	S	R	S	S	MR
Batul <sup>b</sup>	R	R	R	R	R	R
Citrom alma	R	R	R	R	R	R
Kanadai renet	R	R	R	R	MR	R
Máté Dénes	R	R	R	R	R	R
Pónyik alma <sup>2</sup>	R	R	R	R	R	R
Sándor cár	R	R	R	R	R	R
Sikulai <sup>2</sup>	R	R	R	R	R	R
Szabadkai szercsika <sup>2</sup>	R	R	R	R	MS	R
Tordai piros kálvil <sup>2</sup>	R	MS	R	MR	R	R
Vilmos renet <sup>2</sup>	R	R	R	R	R	R

*R* resistant, *MR* moderately resistant, *MS* moderately susceptible, *S* susceptible

<sup>a</sup>Susceptible control

<sup>b</sup>Cultivars previously recommended by Tóth et al. (2005)

**Table 4** The detected markers linked to apple scab resistance genes

	AL07	AM19	AD13	CH02c02a	CH05e03 (165 bp)	OPL19	CH05e03 (173 bp)
Gala <sup>a</sup>	–	–	–	0	–	–	–
Reka <sup>a</sup>	0	–	–	0	0	+	0
<i>M. pumila</i> <sup>a</sup>	–	–	+	+	+	+	–
Prima <sup>a</sup>	+	+	0	0	–	0	–
Batul <sup>b</sup>	–	–	++	++	++	++	–
Citrom alma	–	–	–	–	–	–	–
Kanadai renet	–	–	+	+	+	–	–
Máté Dénes	–	–	–	–	–	+	+
Pónyik alma <sup>b</sup>	–	–	–	–	–	+	+
Sándor cár	–	–	–	–	–	+	+
Sikulai <sup>b</sup>	–	–	–	–	–	–	–
Szabadkai szercsika <sup>b</sup>	–	–	–	–	–	+	–
Tordai piros kálvil <sup>b</sup>	–	–	–	–	–	+	–
Vilmos renet <sup>b</sup>	–	–	–	–	–	+	+

– no resistant allele was found, + heterozygous resistant, ++ homozygous resistant, 0 no evaluation were made or lost data

<sup>a</sup> Susceptible or resistant control

<sup>b</sup> Cultivars previously recommended by Tóth et al. (2005)

The *Vf* gene could not be detected in the investigated old apple cultivars, neither with the AL07 nor AM19 markers. However, the *Vf* resistant control cultivar ‘Prima’ showed the expected result.

Markers linked to the *Vh4* gene were detected in two cultivars. In the ‘Kanadai renet’ and ‘Batul’ cultivars, all the three markers AD13, CH02c02a, and CH05e03 (165 bp), were detected. In addition the *Vh4* gene in ‘Batul’ seems to be homozygous which was also strengthened by the SSR markers.

The markers of *Vh2* gene were detected in seven from ten cultivars, however in the case of ‘Batul’, ‘Szabadkai szercsika’ and ‘Tordai piros kálvil’ only the OPL19 SCAR was shown, the SSR marker CH05e03 (173 bp) could not strengthen the result. In the case of ‘Máté Dénes’, ‘Pónyik’, ‘Sándor cár’, and ‘Vilmos renet’ both marker gave the same result.

## Discussion

Many resistance sources have already been selected from old cultivars, and it is not rare that they show similar field resistance to newly bred scab resistant cultivars (Kellerhals et al. 2012). In our case, the

investigated old cultivars were highly resistant to apple scab and powdery mildew in general. However, no *Vf* resistant cultivar was found by the marker analysis. The *Vf* gene was inherited to apple cultivars through the modern breeding which explains the lack of *Vf* resistant old cultivars. In contrast, the detected markers are suggesting the presence of the *Vh2* and *Vh4* genes. This result is confirmed by the fact that *M. pumila* (Mill.) had an important role in the development of the apple cultivars in the Carpathian Basin (Nagy-Tóth 1998). These results are consistent with the results of other authors (Patzak et al. 2011; Urbanovich and Kazlovskaya 2008) who also stated the absence of *Vf* in old cultivars, while the marker alleles of AD13 and OPL19 were often detected.

In previous studies some of the investigated old cultivars were recommended for organic farming: ‘Batul’, ‘Vilmos renet’, ‘Pónyik’, ‘Sikulai’, ‘Tordai piros kálvil’, and ‘Szabadkai szercsika’ were characterized with acceptable fruit quality and good overall disease resistance (Bálint et al. 2013; Tóth et al. 2004, 2005, 2012).

The suitability of these cultivars in organic orchards is confirmed by the field evaluations and the genetic marker analysis as well. Moreover, ‘Batul’ which has

excellent fruit quality, possibly contains FB\_MR5 fire blight resistance, and considered to be one of the most known old apple cultivars originated in the Carpathian Basin (Tóth et al. 2005; Papp et al. 2011, 2015) is proved to be homozygous *Vh4* genotype, which is often associated with higher degree of resistance, and also bears higher value in the breeding. Using old cultivars as the basis of resistance breeding is a widespread strategy (Kellerhals and Duffy 2006; Lateur and Populer 1994), so hopefully based on our results some of the studied old cultivars, such as ‘Batul’ (*Vh4Vh4*), ‘Vilmos renet’ (*Vh2vh2*), or ‘Kanadai renet’ (*Vh4vh4*), may play a role in the breeding in the future as resistance source. The results are suggesting that some old cultivars have good field resistance against apple scab and powdery mildew; however, the scab resistance could not be explained with the presence of major resistance genes (for example ‘Sikulai’). This suggests that few cultivars might possess polygenic resistance or, with lower chance but possibly, yet unknown major resistance genes.

In the long run, the susceptibility of the cultivars may vary by time, so further field observations are necessary. More objective degree of resistance of the investigated cultivars could only be obtained by evaluating them in untreated conditions. Recently, new apple scab resistance markers have been developed by fine mapping (Cova et al. 2015; Padmarasu et al. 2014). Carrying out analyses with newly developed markers in the future may explain the genetic background of the resistant phenotypes which were lacking the resistance genes in the present study, or clarify the controversial data experienced concerning to the *Vh2* gene. The investigation of the major genes of powdery mildew resistance could be also efficient. The potential of the old apple cultivars which were previously suggested to organic farmers is confirmed by the newer examinations; however, the establishment of test orchards or on-farm experiments would be necessary.

**Acknowledgement** In 2014, The evaluation of the field resistance was carried out in the framework of post-doc research program of the Hungarian Research Institute of Organic Agriculture (ÖMKi).

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