Identification of Apple Scab and Powdery Mildew Resistance Genes in Czech Apple (*Malus* × *domestica*) Genetic Resources by PCR Molecular Markers

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Abstract: The presence of genes for resistance to scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) was studied using molecular markers in a sample of 279 apple cultivars from the Czech collection of apple genetic resources. The sample comprised 37 cultivars supposed to have the *Vf* gene for scab resistance, 97 reference world cultivars and 145 old and local cultivars. Six PCR molecular markers for the scab resistance genes *Vf*, *Vm*, *Vbj*, *Vr* and *Vh* and three PCR molecular markers for the powdery mildew resistance genes *Pl-w*, *Pl-1* and *Pl-d* were used. The marker for the major scab resistance gene *Vf* was detected in all cultivars supposed to have *Vf*, except in Romus 1, and in the three small-fruited cultivars *Malus* Evereste, Golden Gem and Hilleri. The markers of the *Vr* and *Vh* scab resistance genes were detected in 22 cultivars in combination with the marker for *Vf*, in 56 reference world cultivars and in 82 old and local apple cultivars. PCR molecular markers for one or two of the powdery mildew resistance genes were detected in the small-fruited cultivars *Malus* Evereste, Golden Gem, prof. Sprengeri and Hilleri; and in the larger fruited cultivars Hagloe Crab, Borovinka and Tita Zetei. We did not find markers for the scab resistance genes *Vm* and *Vbj* in any of the studied cultivars. They are absent also in the remaining part of the Czech collection of apple genetic resources. PCR molecular markers are useful tools for the identification of resistance genes within apple germplasm collections and can be used to increase the number of sources for disease resistance in breeding programmes.

Keywords: apple; Czech genetic resources; *Malus* × *domestica*; *Podosphaera leucotricha*; powdery mildew; resistance PCR molecular markers; scab; *Venturia inaequalis*

Apples (*Malus* × *domestica* Borkh.) belong to the main fruit species and they are the most important fruit in Europe. Apples are rich in vitamins, minerals, carbohydrates and dietary fibre content. Apples are regularly subjected to infection through filamentous fungi and their infection results in severe losses of production and quality. Most of the commercial apple cultivars are susceptible to fungal diseases, and growers have to apply fungicides 20–30 times per season. The use of resistant cultivars could cut growers' costs and might also contribute to a cleaner environment and to a reduction of fungicide residues on apples for consumers (Soriano *et al.* 2009).

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke) Wint., is one of the most devastating diseases of apples in temperate zones with humid growing seasons. The scab resistance breeding is mainly based on major resistance genes, their pyramiding and combination with polygenic resistance. Various sources of apple scab resistance have been found. Several major scab resistance

genes originated from small-fruited Asiatic *Malus* spp. The genes *Vbj* from *Malus baccata jackii*, *Vb* from *M. baccata*, *Vm* from *M.* × *micromalus* and *M.* × *atrosanguinea* 804, *Vr* from *M. pumila* (Russian seedling) R12740-7A, *Vf* from *M.* × *floribunda* 812 have been introgressed into breeding lines and selections to make them available for breeding purposes. Until now, the *Vf* resistance has mostly been incorporated into commercially available cultivars. The sources of scab resistance are cvs Golden Delicious (*Vg*), Antonovka (*Va*), GMAL 2473 (*Vr2*), Durello di Forli (*Vd*), 1980-015-25 (*Vd3*), Mildew Immune Selection (MIS) (*Vmis*), differential host 2 (*Vh2*) and host 4 (*Vh4*) (derived from *M. pumila* R12740-7A), *M. sylvestris* W193b (*Vh8*) (Gessler *et al.* 2006). Resistance genes *Va*, *Vf* and *Vd3* have been mapped on LG1; *Vbj*, *Vr*, *Vr2*, *Vh2*, *Vh4* and *Vh8* have been mapped on LG2; *Vmis* has been mapped on LG3; *Vd* has been mapped on LG10; *Vb* and *Vg* have been mapped on LG12 and *Vm* has been mapped on LG17 (Soriano *et al.* 2009). Recently, scab resistance genes have been renamed to *Rvi* genes, according to gene-for-gene relationships in the *V. inaequalis-Malus* pathosystem (Bus *et al.* 2009) and currently there are about 17 known genes (Bus *et al.* 2010). PCR molecular markers associated with resistance genes can easily select genotypes with one or multiple resistance genes. Molecular markers have been developed for the identification of resistance genes to scab in the apple genome (Gessler *et al.* 2006). There is a possibility to detect PCR molecular markers of resistance genes *Va* (Hemmat *et al.* 2003), *Vf* (Tartarini *et al*. 1999), *Vm* (Cheng *et al*. 1998), *Vbj* (Gygax *et al*. 2004), *Vr1* (Boudichevskaia *et al.* 2006), *Vh4*, *Vr2* (Patocchi *et al*. 2004), *Vh8* and *Vh2* (Bus *et al.* 2005).

Apple powdery mildew, caused by the fungal pathogen *Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon, is another fungal disease with great impact on fungicide use. Also, it has been found that several major powdery mildew resistance genes originated from small-fruited Asiatic *Malus* spp. Breeders have focused their efforts on introducing the resistance genes *Pl-1* from *Malus robusta* and *Pl-2* from *M. zumi* into traditional breeding lines. Other sources of resistance to mildew are *Pl-w* from White Angel, *Pl-d* from D12 and *Pl-m* from MIS (James *et al*. 2004). Resistance genes *Pl-m* and *Pl-2* have been mapped on LG11; *Pl-d* has been mapped on LG12 and *Pl-w* has been mapped on LG8 (Bus *et al.* 2010). PCR molecular markers have also been developed for the identification of resistance genes to powdery mildew in the apple genome: *Pl-1* (Markussen *et al.* 1995), *Pl-2* (Seglias & Gessler 1997), *Pl-w* (Evans & James 2003) and *Pl-d* (James *et al.* 2004).

The aim of this study was to identify resistance genes to scab and powdery mildew in the collection of Czech apple genetic resources by PCR molecular markers. The determination of resistance genes in apple cultivars and genotypes enlarged the main sources of resistance for breeding programmes.

MATERIAL AND METHODS

Plant material

In our experiment, we used 279 apple cultivars and genotypes in total (Table 2, 3, 4 and 5). One tree of each of 37 *Vf* scab resistant apple cultivars, 97 reference world cultivars and 145 old and local genotypes were selected from apple (1087 cultivars) genetic resources of Research and Breeding Institute of Pomology in Holovousy (CR). To monitor reproducibility and exactness of PCR molecular analyses, we included the second trees of 20 apple cultivars: Ametyst, Goldstar, Rubín, Topaz, Golden Delicious, James Grieve, McIntosh, Resista, Otava, Lord Lambourne, Gala, Spartan, Wealthy, Hetlina, King of Pippins, Edward VII, Böhmischer Jungfernapfel, Čistecké lahůdkové, Rambour Papeleu and Spätblüher Hammerfest.

DNA isolation

Expanded green leaves were collected from June to August (2007–2010) and stored at –45°C until use. Leaves were powdered with liquid nitrogen in pre-cooled mortars. DNA was isolated from approximately 1 g of leaf powder using the SDS isolation method according to Goulao *et al.* (2001).

Molecular resistance marker analysis

Six PCR molecular markers of resistance genes to scab *V. inaequalis* and three PCR molecular markers of resistance genes to powdery mildew *P. leucotricha* were used for molecular analyses (Table 1). Standard PCR protocol (Taq PCR master mix kit, Qiagen, Hilden, FRG), profile and chemicals were used for

Table 1. PCR molecular markers for detection of scab and powdery mildew resistance genes

Marker primers	PCR type	Detected gene	Size (bp)	Reference
VfT F+R	SCAR	Vf	466	Tartarini *et al.* (1999)
AD13-SCAR a+b	SCAR	*Vr1*	950	Boudichevskaia *et al.* (2006)
OPL 19 SCAR a+b	SCAR	*Vh8 + Vh2 = Vr*	433	Bus *et al.* (2005)
Ch02c02	SSR	*Vh4 = Vr2*	176	Patocchi *et al.* (2004)
Vm F+R	SCAR	Vm	687	Cheng *et al.* (1998)
K 08 SCAR a+b	SCAR	*Vbj*	743	Gygax *et al.* (2004)
OPAT 20	RAPD	*Pl-1*	450	Markussen *et al.* (1995)
EMDM 01	SCAR	*Pl-d*	90	James *et al.* (2004)
EM 02 a+b	SCAR	*Pl-w*	250	Evans and James (2003)

PCR – Polymerase Chain Reaction, SCAR – Sequence Characterized Amplified Region, SSR – Simple Sequence Repeat, RAPD – Random Amplified DNA Polymorphism

marker amplification according to authors (Table 1) in Genius thermocycler (Techne, Cambridge, UK) or TGradient thermocycler (Biometra, Goettingen, FRG). Amplification products were resolved via electrophoresis in horizontal 2% agarose gels and visualized by ethidium bromide staining according to Patzak (2001). Amplification products were also resolved via 5% denaturing (8M urea) polyacrylamide gel vertical electrophoresis and visualized by silver-staining (Patzak 2001). Stained and dried gels were duplicated to an opaque daylight film (Promega, Madison, USA). The products were scored for the presence or absence in each sample and recorded by the number of base pairs for each PCR molecular marker based on the size measured with 20 bp DNA Marker (Bio-Rad, Hercules, USA), pGEM DNA marker and 100 bp ladder (Promega, Madison, USA). To monitor reproducibility between runs, we included the above-mentioned duplicated 20 apple cultivars in some runs.

RESULTS AND DISCUSSION

The use of resistant apple cultivars is the best way of eliminating economic losses caused by fungal diseases. For the effective resistance breeding process, it is necessary to identify and evaluate valuable sources of resistance within genetic resources. The use of marker-assisted selection is an excellent instrument for identification of resistance genes and creation of resistance cultivars.

In our first experiment, we identified PCR molecular markers of resistance genes to scab and powdery mildew within 37 *Vf* scab resistant apple cultivars. A PCR molecular marker of *Vf* scab resistance gene was detected in all cultivars (Table 2). The observed results agreed with previously published data on these cultivars (Crosby *et al.* 1992; Fisher *et al.* 2005; Urbanovich & Kazlovskaya 2008; Zoufalá *et al.* 2009). Newly, the *Vf* scab resistance gene was identified in Czech apple cultivars Ametyst, Angold, Biogolden, Dukát, Goldstar, Orion, Opál, Rozela and Hana (Table 2). Zoufalá *et al.* (2009) did not detect the molecular marker of the *Vf* scab resistance gene in cultivars Angold and Dukát. Scab resistant cultivar Angold was derived from the crossing of *Va* scab resistant cultivar Antonovka with cultivar Golden Delicious (Zoufalá *et al.* 2009). *Vf* and *Va* scab resistance genes have been closely mapped on LG1 (Gessler *et al.* 2006) and it is possible that *Vf* molecular marker identified *Va* scab resistance gene or another allele of *Vf* scab resistance gene (e.g. *Vd3* – Soriano *et al.* 2009). Cultivar Dukát was derived from the crossing of cultivars Golden Delicious × Cox's Orange Pippin (Zoufalá *et al.* 2009). But Evans *et al.* (2011) reported that cultivar Dukát was not derived from Golden Delicious based on SSR analysis. Therefore, the scab resistance of cultivar Dukát could be influenced by other ancestors. Next, PCR molecular markers of scab resistance genes *Vr* and *Vh* were detected in 22 *Vf* scab resistant cultivars (Table 2). Our results were identical to published data for previ-

Table 2. Detection of PCR molecular markers of resistance genes within *Vf* scab resistant apple cultivars; plus (+) indicates the presence of a marker

Cultivar	Origin	Scab resistance gene			
		Vf	*Vr1*	*Vr*	*Vr2*
Ametyst	CSK	+			
Angold	CSK	+	+	+	+
Biogolden	CSK	+		+	
Blaník	CSK	+		+	
Dukát	CSK	+			
Ecolette	NLD	+		+	
Florina	FRA	+	+	+	+
Gavin	GBR	+	+		+
Goldstar	CSK	+			
Hana	CSK	+		+	
Jonafree	USA	+			+
Karmína	CSK	+		+	
Kordona	CSK	+	+	+	+
Liberty	USA	+		+	
Mc Free	CAN	+			
Melodie	CSK	+			
NY 58158-2	USA	+			
NY 58553-1	USA	+		+	
Opál	CSK	+			
Orion	CSK	+			
Otava	CSK	+			+
PO 55158-1	USA	+		+	
Priam	USA	+	+		+
Prima	USA	+		+	
Primula	USA	+	+	+	+
Priscilla	USA	+	+	+	+
Rajka	CSK	+			
Red Free	USA	+	+	+	
Relinda	DEU	+			
Resista	CSK	+	+		+
Rewena	DEU	+			
Rosana	CSK	+			
Rozela	CSK	+			
Rubinola	CSK	+			
Selena	CSK	+	+		
Topaz	CSK	+			
Witos	POL	+	+	+	+

Molecular markers of *Vm*, *Vbj*, *PI-w*, *PI-l* and *PI-d* genes were not detected

ously studied cultivars Florina, Jonafree, Otava, RedFree, Relinda, Topaz and Witos (Urbanovich & Kazlovskaya 2008). PCR molecular markers of scab resistance genes *Vm* and *Vbj*, and powdery mildew resistance genes *PI-w*, *PI-l* and *PI-d*, were not detected in any *Vf* scab resistant cultivar.

In our second experiment, we identified PCR molecular markers of resistance genes to scab and powdery mildew within 97 reference world apple cultivars. PCR molecular markers of *Vr* and *Vh* scab resistance genes were detected in 56 apple cultivars (Table 3). The tested reference world apple cultivars (41), without detected molecular markers, are shown in Table 5. We did not detect a PCR molecular marker of *Vf* scab resistance gene in *Vf* scab resistant cultivar Romus 1. Crosby *et al.* (1992) suggested that Romus cultivars could contain only a high level of polygenic resistance and not the *Vf* scab resistance gene. There are also possibilities of the absence of molecular marker on LG1, presence of different allele of *Vf* resistance gene or *Va* resistance gene (Gessler *et al.* 2006; Soriano *et al.* 2009). But the used PCR molecular marker of *Vf* scab resistance gene (Tartarini *et al.* 1999) was not in tight linkage to *Va* resistance gene, which is present in the scab resistant cultivar Produkta, which was not amplified (Table 3). Our results were partly identical to results published by Urbanovich and Kazlovskaya (2008). The results were in accordance with those of the previously studied cultivars Alkmene, Discovery, Golden Delicious, Empire, King Jonagold, Melba, Pinova, Rode Boskoop and Wealthy (Table 3 and 5). The differences were found for scab susceptible cultivars Idared, Elstar and McIntosh (Table 5), although Urbanovich and Kazlovskaya (2008) found a molecular marker of *Vr* (*Vh8* + *Vh2*) resistance gene. PCR molecular markers of scab resistance genes *Vf*, *Vm* and *Vbj*, and powdery mildew resistance genes *PI-w*, *PI-l* and *PI-d* were not detected in all used world apple cultivars.

In our last experiment, we identified PCR molecular markers of resistance genes to scab and powdery mildew within 145 old and local genotypes. PCR molecular markers of scab and powdery mildew resistance genes were detected in 84 apple genotypes (Table 4). The tested old and local genotypes (61), without detected molecular markers, are shown in Table 5. A PCR molecular marker of the *Vf* scab resistance gene was detected in small-fruited *Malus* cultivars: *Malus* Evereste, Golden Gem and Hilleri (Table 4). The cross-reaction

Table 3. Detection of PCR molecular markers of resistance genes within reference world apple cultivars; plus (+) indicates the presence of a marker

Cultivar	Origin	Scab resistance gene		
		Vr1	*Vr*	*Vr2*
Alkmene	DEU		+	
Apollo	DEU		+	
Atlas	CAN	+	+	
Bancroft	CAN	+	+	+
Belréne	FRA		+	
Braeburn	NZL		+	
Britemac	USA	+		
Cox Pomona	GBR		+	
Delicious Richared	USA	+	+	+
Diadém	CSK		+	
Dublet	CSK		+	
Empire	USA		+	
Empire red	USA		+	
Fantasie	POL		+	
Fuji	JPN		+	
Glencross	CAN	+	+	
Glenmary	CAN	+		
Gloster	DEU	+	+	+
Helios	DEU		+	+
Ingrid Marie	DNK		+	
Jamba 69	DEU	+		
Jerseymac	USA	+	+	
Julia	CSK		+	
Julyred	USA	+		
Karin Schneider	DNK		+	
Karmen	CSK		+	
King of the Pippins	GBR		+	
Krasava	CSK		+	
Ligol	POL		+	
Lobo	CAN		+	
Lord Lambourne	GBR	+		
Mantet	CAN	+	+	
Mantet red	CSK	+	+	
Melba	CAN	+	+	
Melba red	CAN	+	+	
Melrose	USA		+	
Mio	SWE		+	
Mollie's Delicious	USA		+	
Oldenburg	DEU		+	

Table 3 to be continued

Cultivar	Origin	Scab resistance gene		
		Vr1	*Vr*	*Vr2*
Oldenburg red	CSK		+	
Orangenburg	DEU		+	
Paulared	USA		+	
Pinova	DEU		+	
Produkta	CSK		+	
Quinte	CAN		+	
Red Spur Delicious	USA	+	+	+
Rode Boskoop	NLD	+		
Rogo	YUG		+	
Romus 1	ROM		+	
Spartan	CAN		+	
Spartan compact	CAN		+	
Stark Earliest	USA	+	+	
Starkrimson Delicious	USA	+	+	+
Vista Bella	USA		+	
Wealthy	USA		+	
Wealthy red	USA		+	

Molecular markers of *Vf*, *Vm*, *Vbj*, *PI-w*, *PI-l* and *PI-d* genes were not detected

between PCR molecular markers of *Vf* resistance gene and *Va* resistance gene was not confirmed, since no molecular markers were amplified in the *Va* scab resistant cultivar Antonovka. PCR molecular markers of *Vr* and *Vh* scab resistance genes were detected most frequently (Table 4). The observed results are original, because no such molecular analysis has been done before. Urbanovich and Kazlovskaya (2008) carried out a similar analysis on 130 apple cultivars and old local genotypes from Belarus genetic resources. Identically, they found PCR molecular markers of *Vr* and *Vh* scab resistance genes in genotypes White Transparent, Winter Banana and Pepin safrannyj. They also found a PCR molecular marker of the *Vr* (*Vh8* + *Vh2*) resistance gene in Antonovka and Borovinka, in contrast to our results (Table 5). Probably, these scab resistance genotypes have included a polygenic resistance, based on another alleles of resistance genes (*Va*, *Vd*, etc.), similarly like Early Victoria, Spätblühender Taffetapfel and Hagloe Crab, which were found by Fisher *et al.* (2005) in German genetic resources. PCR molecular markers of powdery mildew resistance genes were

Table 4. Detection of PCR molecular markers of resistance genes within old and local apple genotypes; plus (+) indicates the presence of a marker

Cultivar	Origin	Scab resistance gene				PM resistance gene		
		Vf	*Vr1*	*Vr*	*Vr2*	*PI-w*	*PI-l*	*PI-d*
Adams Pearmain	GBR			+				
Bergamotova	SUN			+				
Bezjaderka Micurinova	SUN			+				
Bezjaderné	CSK			+				
Bismarck	AUS			+				
Böhmischer Borsdorfer	CSK			+				
Bohnapfel	DEU			+				
Bohušovické	CSK		+					
Borovinka	SUN						+	
Božena Němcová	CSK			+				
Cikánka	CSK			+				
Cusset a´ fruits rouges	SUN			+				
Červený hranáč	CSK		+	+				
Čistecké lahůdkové	CSK		+	+				
Edward VII.	GBR			+	+			
Ellison's Orange	GBR			+				
Fischerovo	BEL		+	+	+			
Funtové	CSK			+				
Gascoyne's Scarlet	GBR			+				
Gestreifter Herbst Calvill	DEU			+				
Graham's Royal Jubilee	GBR			+				
Granátka	CSK			+	+			
Gravenstein	DEU			+				
Grossherzog Friedrich von Baden	DEU			+				
Hagloe Crab	GBR						+	+
Harberts Reinette	DEU			+				
Harris	USA				+			
Hladíkovo přeúrodné	CSK			+				
Chodenapfel	CSK			+				
Jan Říha	CSK			+				
Kaiser Alexander	SUN			+				
Königinapfel	GBR			+				
Kouřimský kropenáč	CSK				+			
Libernáč vinický	CSK			+				
Libovická oranžová reneta	CSK			+				
Lohák	CSK			+				
Malináč Vrchlického	CSK		+	+				
Malus Evereste		+		+	+	+		
Malus Golden Gem		+		+		+		+
Malus Hilleri		+						+
Malus prof. Sprengeri				+		+		
Malus Sikkimensis				+				

Table 4 to be continued

Cultivar	Origin	Scab resistance gene				PM resistance gene		
		Vf	*Vr1*	*Vr*	*Vr2*	*PI-w*	*PI-l*	*PI-d*
Mastná	CSK			+				
Míšeň jaroměřská červená	CSK		+					
Mrázovo z Náhořan	CSK		+	+				
Oberländer Himbeerapfel	NLD			+				
Olivka Žolta	SUN		+	+	+			
Ovčí hubička	CSK		+	+				
Pepin safrannyj	SUN			+				
Podzvičinské	CSK		+					
Pomme Luiken	FRA			+				
Princ Albrecht	DEU			+				
Prinzenapfel	DEU			+				
Proche	CSK			+				
Prostřední	CSK			+				
Rambour Papeleu	SUN			+				
Raskubský zákusek	CSK			+				
Red Astrachan	SUN		+	+				
Red Berlepsch Reinette	DEU			+				
Red Victoria	GBR		+					
Reinette Coulon	BEL				+			
Reinette Grise	FRA				+			
Rose de Boheme	CSK			+				
Rote Sternrenette	BEL			+				
Roter Eiserapfel	DEU			+				
Roter Winterstettiner	CSK				+			
Signe Tillisch	DNK			+				
Smiřické vzácné	CSK		+					
Spätblüher Hein	DEU			+				
Spätblüher Drescher I	DEU			+				
Spätblüher von Bockedra	DEU			+				
Staročeské medové	CSK			+				
Sudeten Renette	CSK			+				
Summer Pearmain	GBR			+				
Štěpánovo z Barchovic	CSK		+					
Táborita	CSK			+	+			
Tita Zetei	ROM		+	+				+
Velišské	CSK			+				
Vinné	CSK			+				
Vršovské růžové zimní	CSK			+	+			
White Astrachan	SUN		+	+				
White Transparent	SUN		+	+	+			
Winter Banana	USA				+			

Molecular markers of *Vm* and *Vbj* genes were not detected; PM – powdery mildew

detected mainly in small-fruited *Malus* cultivars. A PCR molecular marker of the powdery mildew resistance gene *PI-w* was detected in *Malus* Evereste, Golden Gem and prof. Sprengeri; a molecular marker of the powdery mildew resistance gene *PI-l* was detected in cultivars Borovinka and Hagloe Crab; and a molecular marker of the powdery mildew resistance gene *PI-d* was detected in *Malus* Golden Gem and Hilleri and cultivars Tita Zetei and Hagloe Crab (Table 4). PCR molecular mark-

Table 5. A list of studied apple cultivars and genotypes, in which no molecular markers for the studied resistance genes were found

Origin	World apple cultivars	Old and local apple genotypes
AUS	Granny Smith, Granny Smith Spur	
BEL	King Jonagold	Baumann's Reinette, Marbree de Watervliet
CAN	Mc Intosh, Mc Intosh red, Mc Intosh spur, Ontário, Summerred	
CSK	Bohemia, Denár, Desert, Gold Bohemia, Hetlina, Jonalord, Rubín, Šampion, Šampion red, Zlatava	Bláhovo pozděkvěté, Granátové svatodušní, Hájkova muškátová reneta, Hlaváčkovo, Jaroslav Němec, Kostelecké, Košíkové, Kučerovo, Malinové holovouské, Malinové letní, Malinové sloupenské, Mikulášovo, Panenské veliké, Přeloučský šišák, Roter Jungfernapfel, Syreček, Štětínské žluté zimní, Třtické bezejmenné, Vinné hřebíčkové, Zapovězené, Žďárské úrodné
DEU	Bruehahn, James Grieve Neumann's, Pilot	Adersleber Calville, Boiken, Haniger, Holstein, Imperator Wilhelm, Landsberger Reinette, Nathusius Taubenapfel, Purpurroter Cousinot, Spätblühender Taffetapfel, Spätblüher Hammerfest, Spätblüher Drescher II, Spätblüher East Malling, Spätblüher Gaisa, Zuccalmaglios Renette
DNK	Cherry Cox's	
FRA	Lysgolden	Calville d'Automne, Reinette d'Orleans, Reinette de Champagne, Reinette du Canada, Reinette grise de Vignat, Transparente de Croncels, Winter Citronen Apfel
GBR	Cox's Orange Pippin, Discovery, Greensleeves, Imperial all red, James Grieve, James Grieve red, Jester	Blenheim Orange, Cornish Gilliflower, Early Victoria, Golden Noble, London Pippin, Parker's Pippin, Pott's Seedling, Worcester Pearmain
CHE	Arlet, Glockenapfel, Schweizer Orangenapfel	Berner Rosen, Gustav's Daureapfel
NLD	Elstar, James Grieve Lired, Queen Cox's, Royal Gala	Ananas Reinette
NZL	Gala	
POL	Delikates	
SUN		Alma *Malus sieversii,* Antonovka
SWE	Katja	
USA	Estiva, Golden Delicious, Golden Smoothe, Golden Spur, Holiday, Johnson Mc Intosh, Jonagold, Jonamac, Jonared, Macoun, Mc Intosh double red	American Mother, Wagener

ers of scab resistance genes *Vm* and *Vbj* were not detected in all studied genotypes. The sources of these resistance genes, small-fruited *Malus baccata jackii*, *M.* × *micromalus* and *M.* × *atrosanguinea*, have not been incorporated into new apple cultivars (Gessler *et al.* 2006). Because both these original and other small-fruited Asiatic *Malus* spp. were absent in our studied collection of Czech genetic resources, we were not able to detect these molecular markers.

In conclusion, the Czech apple genetic resources show a wide variability of resistance genes to fungal diseases. It is still necessary to create new cultivars with two or more resistance sources to overcome the evolution of pathogen virulence. Newly discovered sources of resistance can be included in resistance breeding programs. The combination of different resistance genes is a possible way of obtaining durable fungal resistance for a long time (Urbanovich & Kazlovskaya 2008). The implementation of molecular markers to the breeding process is very important as they allow to detect necessary genes at different stages of ontogenesis (Gessler *et al.* 2006).

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