The variability of *Venturia inaequalis* CKE. races in the Czech Republic and the accumulation of resistance genes in apple germplasm

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ABSTRACT

The growing of resistant apple varieties against the scab, impacts the *Venturia inaequalis* CKE. races development that can overcome the resistance. For this reason the main breeders object is to cumulate the different genetic mechanisms of resistance against this disease. Presented in this paper is the first genetic study of monosporic isolates in the Czech Republic. By means of RAPD and UPGMA methods which characterised the variability of 10 monosporic isolates from different localities and apple varieties. The monosporic isolate derived from the resistant genotype (*Vf* gene) proved a 79% genetic similarity with the isolate derived from sensitive variety Top Red. The genetic similarity of other isolates did not prove the dependence either on a locality or a host variety. The *Vf* and *Vm* genes accumulation in apple germplasm by means of specific PCR markers was studied. It was confirmed that *Vf* gene donors are always heterozygous. Concurrently it was statistically confirmed that the donor of *Vm* gene (OR-45-T-132) is heterozygous, too. The accumulation of *Vf* and *Vm* major genes against the scab was validated in 25% of seedlings of the cross.

Keywords: apple scab; *Venturia inaequalis*; *Malus* × *domestica*; monosporic isolates; resistance; *Vf* gene; *Vm* gene; RAPD; PCR

During growing, apple varieties with monogenic determined resistance against the scab found five races of *Venturia inaequalis* CKE. which can overcome this resistance. The main object of this paper is the characterisation of the genetic variability of apple scab races in the Czech Republic and the description of selected genes cumulation in apple germplasm.

The apple scab represents one of the most widespread apple diseases, which is caused by ascomycete fungus *Venturia inaequalis*. The pathogen is able to attack all of the tree organs except for the roots. The conidium form of this disease attacks leaves, blooms, fruits and sprouts. The lifecycle of the fungus (saprophytic form) is finished on necrotic fallen leaves. The infection of apples starts at the beginning of spring and the first visible symptoms are localised on the bloom bud scales. By means of conidia the infection begins on leaves, sprouts and fruits. The ascospores of the generative phase, maturing in optimal humidity conditions, are another potential way of infection. These spores arise from perithecium localised on fallen leaves (Tenzer and Gessler 1997).

Molecular genetics techniques are very often used for the evaluation of genetic similarity among various organisms. Williams et al. (1990) developed the RAPD method (Random Amplified Polymorphic DNA) that is based on the amplification of DNA polymorphic fragments. This method is also used for the identification and genetic distance evaluation among different phytopathogenic fungus isolates (Váňová et al. 2000, Vejl et al. 2000). The rDNA variability of different *Venturia inaqualis* races studied by Tenzer et al. (1997) by means of microsatellite markers. Analogous marker variants was also used by Schnabel et al. (1999).

Nowadays, except for chemical protection, the growing of genotypes is used with genetic determined resistance against the scab. The higher resistance, caused by polygenic determined resistance or the presence of *Va* gene from variety Antonovka, is typical for older apple varieties. Resistant breeding by using donors controlling the monogenic resistance is relatively easier. Breeding programmes operate with genes *Vm* and *Vf* most frequently.

The important donor of *Vf* gene is *Malus floribunda* Sieb. – clone 821 (Crosby et al. 1992). Gardiner et al.

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(1995) obtained RAPD markers facilitating detection of part *Malus floribunda* genome that was found in hybrid genotypes and provided their resistance. Gianfranceschi et al. (1996) obtained two RAPD markers for resistance genes against the scab. One of these markers allowed the detection of the main gene of resistance – majorgene *Vf*. This gene was presented in all of the analysed resistant apple varieties. Tartarini et al. developed co-dominant PCR (Polymerase chain reaction) marker unequivocally differencing the dominant and the recessive allele of *Vf* gene. Vejl et al. (2003) published the presence of dominant *Vf* allele in Czech apple varieties genotype as the first.

Dayton and Williams (1970) introduced another dominant gene (*Vm*) also causing the resistance against the apple scab. The donors of this gene are genotypes *Malus* × *atrosanguinea* 804 and *Malus micromalus*. Crosby et al. (1992) published the presence of *Vm* gene in some resistant varieties. Hybrid OR-45-T-132 is often used as the donor of *Vm* gene in apple breeding programmes. Cheng et al. (1998) used RAPD method for the study of *Vm* resistance gene. RAPD marker was converted to dominant STS marker allowing the selection of resistant plants with dominant allele *Vm* gene.

Concurrently with the growing of resistance genotypes a development of new *Venturia inaequalis* races was also confirmed. The aggressivity against apple genotypes with *Vf* allele is typical for these new races. In 1993, a new pathogen race with the pathogenity against genotypes including the *Vf* gene, was identified (Parisi et al. 1993). Urbanietz et al. (1999) found out that this new race is widespread particularly in the Netherlands and in the north Germany and it is able to attack majority genotypes containing *Vf* gene. Parisi and Lespinasse (1999) confirmed these results, too.

MATERIAL AND METHODS

Obtaining and cultivation of *Venturia inaequalis* monosporic isolates. The monosporic isolates were derived from naturally infected leaves of seven apple varieties from four localities of the Czech Republic (Table 1). The leaves were collected in the period of August and September. The conidia were taken up from places of fructifying conidiophores occurrence by means of a sterile spike. The spores were placed on 1 ml of sterile distilled water. The concentration of spores was specified microscopically. The solution of spores was diluted to concentration 3–12 spores per one Petri dish. The obtained colonies were cultivated separately. The agar medium was used for cultivation: 2% malt extract agar, 4% agar, 0.5% mycological peptone. The acidity was modified by citric acid on 4.9 pH. Isolates were cultivated in the darkness; the temperature was 22°C.

Plant material. Five crosses originated from The Research and Breeding Institute of Pomology Holovousy Ltd. were used for the cumulating of the resistance genes study (Table 2). Obtained seeds were after stratification sowed in a greenhouse. 40 seedlings were selected from each of progeny that were tested consecutively by DNA markers. Cultivar Resista and hybrid OR-38-T-16 were used as the donors of dominant allele *Vf*. Hybrid OR-45-T-132 was the donor of allele *Vm*. Hybrid

Table 1. Origin of *Venturia inaequalis* monosporic isolates

Description	Host plants			
	variety	origin	resistance	locality
A	Golden delicious	USA	susceptible	Brno
B	Golden delicious	USA	susceptible	Crhov
C	Golden delicious	USA	susceptible	Žabčice
D	Golden delicious	USA	susceptible	Rapotice
E	HL1196	Czech Republic	resistant	Brno
F	Ruclíva	Czech Republic	susceptible	Brno
G	Croncels	France	susceptible	Crhov
H	Top Red	USA	susceptible	Žabčice
I	Šampion	Czech Republic	susceptible	Žabčice
J	HL1047	Czech Republic	susceptible	Žabčice
K	Standard monosporic isolate (Collection of microorganisms Masaryk University in Brno)			

Table 2. Analysed crosses and segregation of *Vf* gene marker

Parental progeny		Theoretical ratio of F_1	Real ratio of F_1	χ^2	Expectation *P*
Maternal genotype	Paternal genotype				
OR-45-T-132 *vfvf*	HL782 (Rubin × Priscila) *vfvf*	uniformity *vfvf*	uniformity *vfvf*	–	1
OR-45-T-132 *vfvf*	HL17 (Zvonkové × Šampion) *vfvf*	uniformity *vfvf*	uniformity *vfvf*	–	1
HL665 (Spartan × Antonovka) *vfvf*	OR-45-T-132 *vfvf*	uniformity *vfvf*	uniformity *vfvf*	–	1
OR-45-T-132 *vfvf*	Resista *Vfvf*	50% of *Vfvf* 50% of *vfvf*	37.5% of *Vfvf* 62.5% of *vfvf*	1.6	(0.1–0.3)
Resista *Vfvf*	OR-38-T-16 *Vfvf*	25% of *VfVf* 50% of *Vfvf* 25% of *vfvf*	0% of *VfVf* 65% of *Vfvf* 35% of *vfvf*	–	0

HL655 is probably the donor of *Va* gene from cultivar Antonovka.

DNA isolation from *Venturia inaequalis* monosporic isolates. The colonies (diameter 6–8 cm) were homogenised in liquid nitrogen. DNA was isolated by GeneElute Plant Genome DNA Kit (Sigma, Germany).

DNA isolation from apple fresh leaves. The leaves collection was proved in September at daily temperature 20°C. Collected leaves were put into an icebox immediately. After three hours, the leaves were fixed by liquid nitrogen and DNA was isolated from them by set GeneElute Plant Genome DNA Kit (Sigma, SRN).

RAPD analysis of variability of *Venturia inaequalis* monosporic isolates. The sequences of used RAPD decameric primers are showed in Table 3. The RAPD composition was as follows: template DNA 30 ng/25 µl, primer 30 ng/25 µl, *Taq* polymerase (Fermentas, Litva) 0.7 U/25 µl, 0.2mM dNTP, 2.5mM $MgCl_2$. The following profile was used for the amplification: 1 cycle (94.0°C/180 s, 36.0°C/45 s, 72.0°C/60 s), 40 cycles (94.0°C/20 s, 36.0°C/45 s, 72.0°C/60 s) and 1 cycle (72.0°C/360 s). The RAPD markers were separated on horizontal electrophoresis in 1.5% agarose gel at constant 3.3 V per 1 cm clearance between electrodes over 150 minutes. Amplified RAPD fragments were stained by ethidium bromide (Sambrook et al. 1989). The program GelManager for Windows (BioSystematica, Great Britain) was used to evaluate of the variability between isolates. Only polymorphic RAPD bands were used for Dice's coefficients computing (Jackman 1994, Vejl et al. 2000). UPGMA (Unweighted Pair

Table 3. Sequences of RAPD primers and number of the polymorphic bands

Primer	Sequence	Number of the polymorphic bands	Size range of polymorphic bands (bp)
OPG03	5′ GAG CCC TCC A 3′	12	1650–510
OPG05	5′ CTG AGA CGG A 3′	14	3000–380
OPG17	5′ ACG ACC GAC A 3′	12	2300–500
OPH04	5′ GGA AGT CGC C 3′	15	2200–500
OPN11	5′ TCG CCG CAA A 3′	14	2200–330
M2	5′ GCC ACA CAC A 3′	18	3500–340
RAPD1	5′ ACG CAG GCA C 3′	16	2100–400
P49	5′ GTA CCA GTG A 3′	9	3000–950

Table 4. Analysed crosses and segregation of *Vm* gene marker

Parental progeny		Theoretical ratio of F_1	Real ratio of F_1	χ^2	Expectation *P*
Maternal genotype	Paternal genotype				
OR-45-T-132 *Vm-*	HL782 (Rubin × Priscila) *vmvm*	50% of *Vmvm* 50% of *vmvm*	45.0% of *Vmvm* 55.0% of *vmvm*	0.4	(0.5–0.7)
OR-45-T-132 *Vm-*	HL17 (Zvonkové × Šampion) *vmvm*	50% of *Vmvm* 50% of *vmvm*	52.5% of *Vmvm* 47.5% of *vmvm*	0.1	(0.5–0.7)
HL665 (Spartan × Antonovka) *vmvm*	OR-45-T-132 *Vm-*	50% of *Vmvm* 50% of *vmvm*	57.5% of *Vmvm* 42.5% of *vmvm*	0.9	(0.3–0.5)
OR-45-T-132 *Vm-*	Resista *vmvm*	50% of *Vmvm* 50% of *vmvm*	52.5% of *Vmvm* 47.5% of *vmvm*	0.1	(0.5–0.7)
Resista *vmvm*	OR-38-T-16 *vmvm*	uniformity *vmvm*	uniformity *vmvm*	–	1

Group Method using Averages) cluster analysis was used for dendrogram construction.

Co-dominant PCR markers of the *Vf* gene. The primers for Multi PCR according to Tartarini et al. (1999) were used for the detection of *Vf* gene allelic constitution. The reaction and amplification conditions of PCR for analyses were modified according to Vejl et al. (2003).

Dominant PCR markers of the *Vm* gene. The primers pair according to Cheng et al. (1998) was used for the amplification of dominant marker. PCR conditions were modified: template DNA 25 ng/25 µl, forward primer 0.2µM, reverse primer 0.2µM, Taq polymerase 0.8 U/25 µl, dNTP 0.2mM, $MgCl_2$ 1.5mM. The following profile was used for the amplification: 35 cycles (94.0°C/30 s, 57.5°C/60 s, 72°C/60 s) and 1 cycle (72°C/480 s). PCR markers were analysed by electrophoresis in 1% agarose gel in Tris-borate-EDTA buffer (Sambrook et al. 1989).

RESULTS

Obtaining and cultivation of *Venturia inaequalis* monosporic isolates. Ten monosporic isolates were obtained according to described selected methods

Table 5. Dice's coefficients of genetic similarity (%) between monosporic isolates *Venturia inaequalis*

A	100										
B	39	100									
C	22	39	100								
D	51	19	30	100							
E	15	30	24	27	100						
F	31	45	56	33	22	100					
G	23	43	57	29	22	89	100				
H	16	32	25	21	79	17	16	100			
I	19	40	47	23	18	71	75	13	100		
J	23	41	45	24	22	69	76	13	91	100	
K	18	16	10	19	17	18	78	11	17	26	100
	A	B	C	D	E	F	G	H	I	J	K

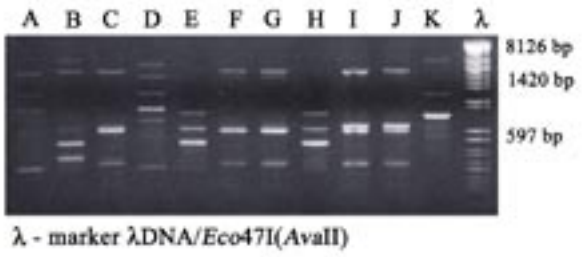

Figure 1. Electrophoreogram of RAPD marker – OPN11 of *Venturia inaequalis* monosporic isolates

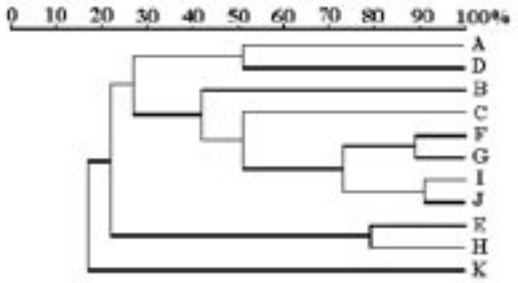

Figure 2. Dendrogram of the analysed *Venturia inaequalis* monosporic isolates, compiled on the basis of Dice's similarity coefficients

that were consecutively cultivated. Approximately after 40 days of cultivation all isolates began to create conidia.

DNA isolation. The quality of DNA was obtained in all of monosporic isolates. Average quantity was 72 μg/1 g mycelium. The coefficient of variation was 8%. Undamaged DNA was isolated also from plant tissues. Average quantity was 42 μg/1 g fresh leaves. The coefficient of variation was 11%. In both cases DNA was successfully used for PCR analysis.

RAPD analysis of variability of *Venturia inaequalis* monosporic isolates. There were tested 80 decameric primers. Only 8 primers provided isolate-specific RAPD bands, which were not affected by the occurrence of unspecific amplifications. The characteristic of RAPD polymorphic bands is presented in Table 3. Figure 1 presents typical electrophoreogram of RAPD markers by using primers OPN11. The different inoculations of individual isolates were evaluated by RAPD method; all RAPD profiles were identical. The overview of Dice's coefficients of similarity between individual RAPD profiles is shown in Table 5. The highest genetic similarity on the value 91% was registered between isolates obtained from genotypes Šampion (I) and HL1047 (J), respectively, locality of Žabčice. The data is shown in Table 5 and Figure 2. A high similarity (89%) of RAPD profiles shows also pair of isolates from Ruclíva (Brno) (F) and Croncels (Crhov) (G). Isolate E from resistant genotype HL1196 showed relatively high similarity to isolate H from Top Red variety (Žabčice). The lowest genetic similarity (11%) was discovered between standard isolate K and isolate H. The only two isolates A (Brno) and D (Rapotice) of all derived from Golden Delicious variety showed more genetic similarity on the level of 51%. The dendrogram cannot show the influence of the locality to similarity of isolate RAPD profiles.

Co-dominant PCR markers of *Vf* gene. All parental components and their progenies were evaluated in the view of the allelic constitution of *Vf* gene. From dataset, shown in Table 2, results that the donors of *Vf* gene (Resista and OR-38-T-16) were used in two different hybrid combinations. The marker in these combinations routinely segregated, in other progenies was not identified (Figure 3).

Dominant PCR markers of *Vm* gene. Optimal conditions for the amplification of 687 bp characteristic product in OR-45-T-132 were created during

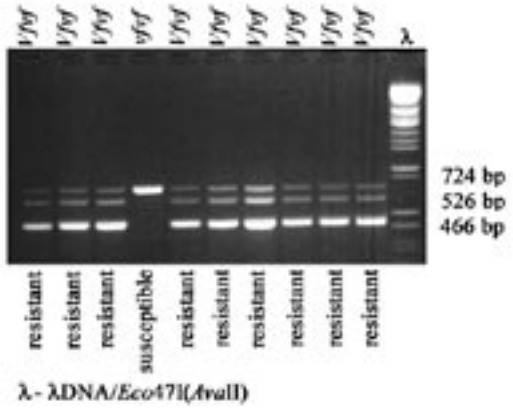

Figure 3. Segregation of co-dominant marker of *Vf* gene in cross OR-45-T-132 × Resista

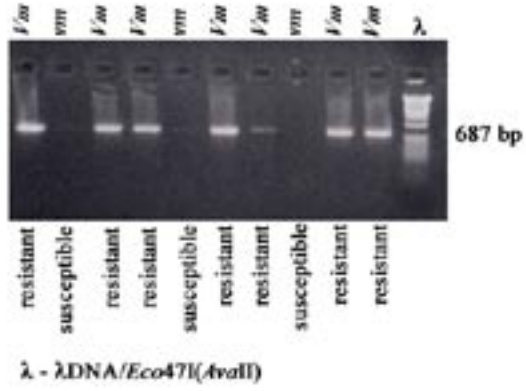

Figure 4. Segregation of dominant marker of *Vm* gene in cross OR-45-T-132 × Resista

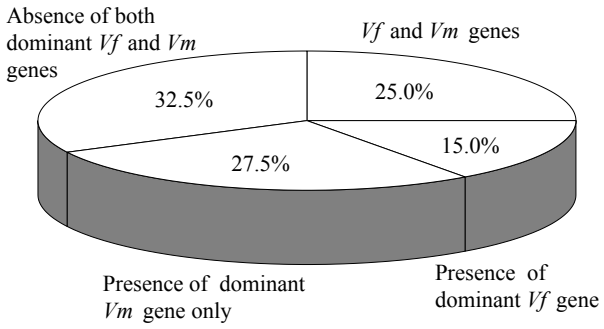

Figure 5. *Vf* and *Vm* gene combinations in cross OR-45-T-132 × Resista

optimisation. This genotype is the only source of *Vm* at our disposal in the Czech Republic. This marker also is very specifically amplified in four crosses where the donor was used like a parent (Table 4 and Figure 4).

Cumulation of *Vf* and *Vm* genes. The cross OR-45-T-132 × Resista was used for the study of cumulation of both genes (Figure 5). From the graph flows show that 67.5% of progeny had at least one of *Vf* and *Vm* major gen pair. In 25% of progeny were concurrently cumulated both resistance genes.

DISCUSSION

One of the factors impacting reproducibility of RAPD analysis results is DNA isolation and purification method. GeneElute Plant Genome DNA Kit (Sigma, Germany) originally designed for plant DNA isolation was proved as suitable for the isolation from mycelium, too. In comparison with laboratory extraction methods (Schnabel et al. 1999, Tenzer et al. 1999), this type of isolation is faster and provides the higher stability of conditions. The DNA amount, acquired from one colony of isolate, corresponds to amount of other phytopathogenic fungus (Vejl et al. 2000).

Obtained results described the variability of *Venturia inaequalis* races can be consider to be the first molecular analysis of this pathogen in the Czech Republic. For this reason it is difficult to compare these results with the conclusions of Schnabel et al. (1999), who evaluated the variability of races in the USA. Also Tenzer et al. (1999) evaluated the variability of an entirely different European race of *Venturia inaequalis*. The important result of this paper is one monosporic isolate derivation (isolate E) that overcame HL1196 hybrid resistance including *Vf* gene. Occurrence *Venturia inaequalis* races attacking the genotypes containing *Vf* gene were noticed by Parisi et al. (1993) and Parisi and Lespinasse (1999) in Western Europe. Also Urbanietz et al. (1999) has found that this new race is expanded in the Netherlands and in the north Germany. The occurrence of this new race is probably high also in the Czech Republic. The isolate (E) was derived from contaminated leaves in the Brno locality. The value of Dice's coefficient between isolate E and isolate H (sensitive variety Top Red from Žabčice) was calculated at 79%. The distance between localities Brno and Žabčice is approximately 20 km, and so it can be supposed that there is the possibility of spores transferring between these two localities. From the high genetic similarity, it is obvious that this race is able to attack both sensitive and resistant (*Vf*) genotypes.

The highest genetic similarity (91%) was assessed between isolates I (Šampion) a J (HL1047). The high genetic similarity of these isolates is probably caused by the same locality (Žabčice).

The progenies of five crosses were used for the study of the different genetic mechanisms of resistance against the apple scab. The DNA of all evaluated genotypes was successfully isolated by means of GeneElute Plant Genome DNA Kit (Sigma, Germany). Vejl et al. (2003) presented that this method did not offer quality DNA for analysis. It is possible that the reason of successful DNA isolation by means of GeneElute Plant Genome DNA Kit (Sigma, Germany) was the lower temperature (15°C) in the greenhouse in the day of sample collection. The amplification results of co-dominant marker *Vf* gene (Tartarini et al. 1999) corresponded with the results of modified method according to Vejl et al. (2003). The absence of dominant homozygous *VfVf* in cross Resista × OR-38-T-16 progeny can be the result of lower evaluated seedlings number than Vejl et al. (2003) have used.

The experiments according to Cheng et al. (1998) were used for the detection of dominant gene *Vm*. The amplification conditions according to Cheng et al. (1998) were not able to ensure the evident specificity of 687 bp fragment amplification. Large optimisation of reaction composition and the time-temperature profile PCR was made for these reasons. A modified method was able to serve as the reliable amplification of the dominant marker only in *Vm* gene donor (OR-45-T-132) and its progenies. The cited author also has used the genotype and has discovered the fact that this hybrid is the reliable donor of *Vm* gene. The designed marker is able to detect presence only of the dominant allele. For these reasons the statistical evaluation was made on four crosses of donor OR-45-T-132 with different genotypes without the gene *Vm* (Table 4). In the case of three crosses, the real segregation ratio was coincident with the segregation ratio of the backcross in statistical expectation $p \in (0.5; 0.7)$. Hybrid OR-45-T-132 is evidently heterozygous.

Discussed markers of *Vf* and *Vm* genes were also used for the study of cumulation both majorgenes

in one individual. The crossing of OR-45-T-132 and Resista (*vfvfVmvm* × *Vfvfvmvm*) were the only combinations for the study of both genes accumulated (Tables 2 and 4). The absence of the linkage between these genes is a precondition for the constitution of 25% *Vf-Vm*-genotypes. This presumption was, although most curious, precisely confirmed in the molecular genetic way.

Accurately 25% of the seedlings have proved heterozygous constitution *Vfvf* and the presence of dominant marker *Vm* (Figure 5). Hybrid HL665, derived from variety Antonovka, it is possible to consider it as the donor of *Va* gene or polygenic factors. The quantitative resistance genes and majorgene *Vm* are combined in the progeny of crossing HL665 × OR-45-T-132. Since there have not been known any STS *Ver* gene markers up until today, it was not possible to accomplish the molecular genetic evaluation of accumulation of these genes.

REFERENCES

Cheng F.S., Weeden N.F., Brown S.K., Aldwinckle H.S., Gardiner S.E., Bus W.G. (1998): Development of DNA marker for *Vm*, a gene conferring resistance to apple scab. Genome, *41*: 208–214.

Crosby J.A., Janick J., Pecknold P.C., Korban S.S., O'Connor P.A., Ries S.M., Goffreda J., Voordeskers S. (1992): Breeding apples for scab resistance 1945, 1990. Acta Hort., *317*: 43–70.

Dayton D.F., Williams E.B. (1970): Additional allelic genes in *Malus* for scab resistance of two reaction types. J. Am. Soc. Hort. Sci., *95*: 735–736.

Gardiner S., Bus V., White A., Noiton D., Bassett H., Rikkernik E., Ball R., Guilford P., Forster R. (1995): A genetic map around the V_f gene for resistance to apple scab. Plant Genome IV. Conf., San Diego: 227.

Gianfranceschi L., Koller B., Seglias N., Kellerhals M., Gessler C. (1996): Molecular selection in apple for resistance to scab caused by *Venturia inaequalis*. Theor. Appl. Genet., *93*: 199–204.

Jackman P.J.H. (1994): GelManager for Windows. Version 1.5. User's Reference. Biosystematika, U.K.

Parisi L., Lespinasse Y. (1999: Pathogenicity of a strain of *Venturia inaequalis* race 6 strains on apple clones (*Malus* spp.). Acta Hort., *484*: 443–447.

Parisi L., Lespinasse Y., Guilaumes J., Kruger J. (1993): A new race of *Venturia inaequalis* virulent to apples with resistance to the V_f gene. Phytopathology, *83*: 533–537.

Sambrook J., Maniatis T., Fritsch E.F. (1989): Molecular cloning. A laboratory manual. 2nd ed. Cold Spring Harbour Laboratory Press.

Schnabel G., Schnabel E.L., Jones A.L. (1999): Characterization of ribosomal DNA from *Venturia inaequalis* and its phylogenetic relationship to rDNA from other tree-fruit *Venturia* species. Phytopathology, *89*: 100–108.

Tartarini S., Gianfransceschi L., Sansavini S., Gessler C. (1999): Development of reliable PCR markers for the selection of the V_f gene conferring scab resistance in apple. Plant Breed., *118*: 183–166.

Tenzer I., Degli Ivanissevich S., Morgante M., Gessler C. (1999): Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. Phytopathology, *89*: 748–753.

Tenzer I., Gessler C. (1997): Subdivision and genetic structure of four populations of *Venturia inaequalis* in Switzerland. Eur. J. Plant Pathol., *103*: 565–571.

Urbanietz A., Schmidt H., Dunenmann F. (1999): Molecular markers in early seedling tests for scab and mildew in apples. Acta Hort., *484*: 429–434.

Váňová M., Polišenská I., Vejl P., Skupinová S. (2000): Differentiation of cereal eyespot fungus using morphologic characteristic and PCR diagnostic tests. Plant Prot. Sci., *36*: 57–64.

Vejl P., Skupinová S., Blažek J., Sedlák P., Bardová M., Drahošová H., Blažková H., Milec Z. (2003): PCR markers of apple resistance to scab (*Venturia inaequalis* CKE.) controlled by *Vf* gene in Czech apple breeding. Plant Soil Environ., *49*: 427–432.

Vejl P., Skupinová S., Polišenská I., Váňová M. (2000): Distinguishing isolates of cereal stem disease pathogens of the genus *Tapesia* from isolates of *Fusarium* and *Rhizoctonia* using the RAPD method. Plant Prot. Sci., *36*: 132–140.

Williams G.K., Kubelik A.R., Livak K.L., Rafalski J.A., Tingey S.V. (1990): DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res., *18*: 6531–6535.

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ABSTRAKT

Variabilita ras *Venturia inaequalis* v ČR a kumulace genů rezistence v genofondu jabloní

Pěstování odrůd jabloní rezistentních vůči strupovitosti ovlivňuje vývoj ras *Venturia inaequalis* CKE., které mohou tuto rezistenci překonávat. Z těchto důvodů je cílem šlechtitelů kumulovat různé genetické mechanismy rezistence vůči této chorobě. Je prezentována první genetická studie monosporických izolátů *V. inaequalis* v ČR. Metodou RAPD a UPGMA analýzy byla charakterizována variabilita deseti monosporických izolátů z různých lokalit a odrůd jabloní. Monosporický izolát odvozený z rezistentního genotypu (*Vf* gen) vykazoval 79% genetickou podobnost s izolátem

odvozeným ze senzitivní odrůdy Top Red. Genetická podobnost ostatních izolátů nevykazovala závislost na lokalitě odběru ani na hostitelské odrůdě. Pomocí specifických PCR markerů byla studována kumulace *Vf* a *Vm* genů v genofondu jabloní. Bylo zjištěno, že donory genu *Vf* jsou vždy heterozygoti. Statisticky bylo potvrzeno, že donor *Vm* genu (OR-45-T-132) je rovněž heterozygot. Kumulace *Vf* a *Vm* majorgenů rezistence vůči strupovitosti byla potvrzena u 25 % potomků jednoho křížení.

Klíčová slova: strupovitost jabloní; *Venturia inaequalis*; *Malus* × *domestica*; monosporické izoláty; rezistence; gen *Vf*; gen *Vm*; RAPD; PCR

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