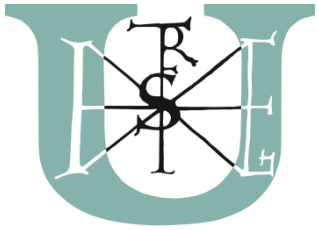

Szent István University

Thesis of doctoral (PhD) dissertation

Characterization of the disease resistance of apple cultivars and breeding materials by phenotyping and molecular marker analyses

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1. Background of the study, research goals

1.1. Some aspects of the international and Hungarian apple resistance breeding

The domesticated apple (*Malus* × *domestica* Borkh.) is the most relevant fruit species of the temperate zone. Cosmopolitan cultivars, which are typically susceptible to the main diseases of apple, dominate the market worldwide. On the one hand the plant protection treatments applied to grow these cultivars have enormous costs, on the other hand they have considerable environmental impact. Resistant apple cultivars offer a solution for these problems, thus were started to be developed in the previous century; and for today the main aim of apple breeders is to achieve disease resistance and high fruit quality of the new cultivars (Laurens 1998; Sansavini et al. 2004).

Apple scab (*Venturia inaequalis* Cke./Wint.), fire blight (*Erwinia amylovora* Burrill) and powdery mildew (*Podosphaera leucotricha* Ell. et Ev./Salm.) are the three main diseases of apple. Since the breeding work started, breeders were focusing on the apple scab resistance; however, powdery mildew resistance had also appeared among the breeding goals, in the beginning of the 20th century. Although, the causal agent of fire blight did only reach Europe in 1958 (Peil et al. 2009), the breeding work pays great attention to fire blight resistance, due to the lack of resistant cultivars, and attainable plant protection treatments.

In Hungary, apple breeding program was first started in the Fruit Growing Department of the University of Horticulture and Food Technology (later Department of Pomology of BCU or more later SZIU) in 1960, which aimed to develop apple cultivars with differing ripening time, good fruit quality, and powdery mildew resistance (Kovács, 1985). The resistance breeding program of the Dept. was renewed in the beginning of the 90’s, and the new aims became to reach disease resistance against multiple diseases, and excellent

fruit quality (Tóth et al. 1994). As a result of the work, four multiresistant cultivars (‘Artemisz’, ‘Cordelia’, ‘Hesztia’, ‘Rosmerta’) and two tolerant cultivars (‘Rodonit’ és ‘Karneol’) were registered in Hungary, which later two are suggested for integrated production (Tóth et al. 2012; Tóth, 2016).

1.2. Relevance of the old apple cultivars from the Carpathian basin

The basis of the resistance breeding work is to explore the genotypes which are applicable gene sources. Besides the modern cultivars, the wild apple species are considerable gene sources of the breeding, as well as those old apple cultivars, which are not cultivated for today. Thus within the frame of the Hungarian breeding work the old apple cultivars of the Carpathian basin were collected and preserved in the gene bank of Soroksár (Tóth, 2005). Protecting the old cultivars, and involve them to the breeding is in line with the international guidelines, furthermore, it helps to protect the biodiversity and cultural values of the countryside.

The topic of biological diversity (biodiversity) is getting increasing attention at the international level, and indirectly includes the diversity of cultivated fruit species. The actual program (”Magyarország élelmiszergazdasági programja 2016-2050”) published by the Hungarian Ministry of Agriculture (2016) does share many of the base ideas of the Rio Conference held in 1992, and aims to enhance the biological diversity in Hungary.

1.3. The genetic background of disease resistance in apple

In general, it is typical to the host-pathogen relationship that resistance genes (*R* genes) do evolve in the host, and effector coding avirulence genes (*Avr* genes) develop in the pathogen as a result of their coevolution, and thus there is a specific relationship between these genes.

The relationship between the *R* and *Avr* genes was first described by Flor (1942), who called it gene-for-gene (GFG) relationship. Deriving from the GFG hypothesis, the resistance coded by the *R* genes is race specific and vertical (Vanderplank, 1963). So, it could be said, that according to the modern implication, the *R* genes are "polymorphic plant genes that control gene-for-gene disease resistance" (Bent and Mackey, 2007).

The GFG relationship was confirmed in the *Malus*-*Venturia* system among the first attempts (Williams and Shay, 1957), and later qualitative resistance of monogenic inheritance was also observed in the case of powdery mildew (Krieghoff, 1995), which can be broken down by the varied pathogen races. Fire blight resistance has long been considered to be a complex polygenic trait in the literature. However, newer studies have confirmed the presence of GFG relationship first described by Flor, also in the case of the *Malus-Erwinia* system. With other words, the resistance of the host was solely regulated by one race-specific major gene (monogene) (Vogt et al. 2014).

1.4. Distribution of the pathogen races which are threatening apple production

So, the efficiency of race-specific disease resistance is closely related to the composition of pathogen race virulence occasionally responsible for the infection pressure. The occurrence of pathogen races should be assessed in order to evaluate the field-resistance precisely, meanwhile, the assessments might draw light upon serious epidemic risks. The most typical example of this phenomena affecting apple production, is the occurrence of scab race 6, which hugely undermined the trust towards resistant apple cultivars (Parisi et al. 1993). Few studies are dealing with the distribution of powdery mildew and fire blight, in contrast the apple scab races are extensively being monitored in our days, mainly in Europe, but all around the world (Bus et al. 2011).

1.5. The possible ways of monitoring resistance genes

Earlier the classical breeding and cultivar evaluation was exclusively based on phenotype assessment. However, in the case of many traits phenotyping is very expensive and/or takes a lot of time to be carried out. In order to ascertain the presence of the precise *R* gene coding race-specific resistance on the basis of the phenotype, artificial infections can be made with a set of pathogen strains with various virulence. In some cases this is a suitable option (Bus et al. 2005), but the method is surely not efficient enough to be used in the breeding.

Otherwise technically unattainable data can be gained from molecular examinations (Collard and Mackill, 2008). The presence of a proper gene in the genotypes can be monitored in a fast and efficient way using molecular marker analyses. In favor of the marker assisted breeding (MAB) many scientific publications are dealing with the mapping of resistance genes, and with the development of molecular markers suitable for tracking their inheritance.

1.6. Research goals

1. Evaluating the scab and powdery mildew resistance of old apple cultivars on the field

2. Identifying *Venturia inaequalis* pathogen races from Hungary

3. Characterization of the scab, powdery mildew mildew and fire blight resistance of old apple cultivars, newly bred Hungarian cultivars and breeding materials by molecular marker analyses.

4. Selection of new gene sources and carry out further genetic analyses on them

2. Materials and methods

2.1. Location of the research

The field research was carried out on the experimental fields of the SZIU Fruit Growing Dept. in Soroksár. Integrated plant protection methods were used on the trees of the modern cultivars, and old apple cultivars located in the collection of historical cultivars. No plant protection treatment was applied on the resistant cultivar collection in a relevant amount.

The genetic examinations were made in the laboratory of molecular biology of the SZIU Fruit Growing Dept. Some steps of the research (fragment lenght analyses, sequencing) were accomplished through labor service.

2.2. Examined cultivars

In the frame of our research work we examined 15 *Venturia* race indicator cultivars, 57 old Hungarian cultivars, and 12 genotypes developed by the Hungarian breeding work. Furthermore, reference cultivars were used for the regarding examinations.

2.3. Field research

We examined the scab and powdery mildew resistance of 57 old apple cultivars from the Carpathian basin. Two trees per cultivar were examined, and we used bonitation tables containing incidence values for apple scab and powdery mildew (table 1). The 15 indicator cultivars were examined for eight years (2009-2016), to monitor the *Venturia inaequalis* races. Assessments were made two times per year. Only the actively sporulating *Venturia* lesions were taken into account in the case of scab, while the detection of powdery mildew infection were made on the basis of infected shoot tips.

Table 1. The scale used for measuring the degree of scab/powdery mildew resistance, according to Király et al. (2015)

Degree of resistance	Leaves with active sporulation (pcs)/100 leaves (pcs)	Shoots infected with powdery mildew (pcs)/50 shoots (pcs)	Scale
Resistant	0	0	0
Moderately resistant	1-2	1-2	1
Moderately susceptible	3-10	3-5	2
Susceptible	10-100	5-50	3

The meteorological data was collected by an iMETOS® system (distributed by Agrárin Kft. Szőlősgyörök, Hungary). Based on the meteorological parameters we calculated the number of Mills periods, which describes the risk of scab infection (Mills, 1944, MacHardy és Gadoury, 1989).

2.4. Molecular research

Buds or young leaves were collected on the Soroksár Research Station for DNA extraction. The DNA was extracted using QIAGEN DNeasy® Plant Mini kit (Hilden, Germany), or E.Z.N.A.® Plant DNA kit (Norcross, USA).

Altogether 20 SSR and SCAR primers were used. The old cultivars from the Carpathian basin were analyzed with 15 markers associated with scab, powdery mildew, or fire blight. We tested the cultivars/genotypes produced by the Hungarian breeding work with a different set of 14 markers linked to scab or fire blight. We ordered the primers from the Sigma-Aldrich® Kft. (Budapest, Hungary).

The DNA fragments were amplified by an Applied Biosystems (Foster City, USA) Thermal Cycler 2720 type PCR instrument. DreamTaq™ Green PCR Master Mix (2×) kit (Fermentas, Waltham, USA) was used for the PCR. The final volume of the PCR product was 16 µl. The program of the PCR was always adjusted according to the regarding literature, with slight modifications.

The PCR products of the SCAR primers were analyzed on agarose gel. The program ran 120 V, for 20 or 40 minutes. The samples were dyed with GR Safe® (Lab Supply Mall, Gaithersburg, USA). The evaluation of the samples were carried out under UV light using GeneRuler™ (Thermo Fisher Scientific, Waltham, USA) DNA ladder (1kb).

The detection of the fragment lengths, and the sequencing was made by the Biomi Kft. (Gödöllő, Hungary) with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA) instrument. The SSR data was analyzed with the Peak Scanner 2.0 (Thermo Fisher Scientific, Waltham, USA) software. The sequence reads were analyzed with GeneStudio™ Professional 2.2 (www.genestudio.com) software.

2.5. Statistical methods

The basic statistics were made with the PASW Statistics 18 (SPSS®) and Microsoft Excel v. 14.0 (Microsoft®) software.

3. Results

3.1. Scab and powdery mildew resistance of old apple cultivars

The apple scab and powdery mildew resistance of 57 old apple cultivars were examined on the field for six years. We evaluated the apple scab and powdery mildew incidence values together with the related climatic parameters, and statistical data analysis was also made. Calculating the infection risks based on the common methods used in the plant protection practice, we found close relation between the expected risks and the actual incidence values of the regarding diseases, in the whole research period. Similarly, we found that the incidence values of both pathogens can easily be described with exponential curve, which is the expected distribution, confirming that the data collection was appropriate. Data of artificial infections previously made in the Dept. with *Erwinia* was used to complete our data, in order to evaluate the disease resistance against all the three main diseases of apple. As a result of all of this, 10 cultivars were resistant against all three diseases (scab, powdery mildew, and fire blight): ‘Angyal Dezső’, ‘Batul’, ‘Damjanich’, ‘Dániel féle renet’, ‘Szemes alma’, ‘Pónyik’, ‘Sikulai alma’, ‘Szabadkai szercsika’, and ‘Vilmos renet’.

3.2. Distribution of *Venturia* races

The occurrence of *Venturia inaequalis* races (race 1-7) in Hungary was monitored by evaluating the scab infections of 15 indicator cultivars on the field, for eight years (table 2.). The distribution of pathogen races may greatly vary with time and place, so we also collected data and samples from farmers, regarding the most important races (race 6 and 7). Based on all of this, the presence of *Venturia* races 1, 3 and 5 in Hungary is proved, considering that the field study gave clear results in their cases. No data suggested the presence of race 4, while the presence of race 2, 6 and 7 is still questionable, as nor the quality or quantity of the data suggesting their presence is sufficient.

Table 2. Incidence of scab infection (0-3) on race indicator cultivars (Soroksár, 2009-2016)

Cultivar	*R* gene[1]	*R* gene (new)[2]	2009	2010	2011	2012	2013	2014	2015	2016
Gala	-	-	3	3	3	3	3	3	3	3
Golden Delicious	*Vg*	*Rvi1*	3	3	3	3	3	3	3	3
Reka	*Vh2*	*Rvi2*	0	3	0	0	0	0	0	0
Malus 'Geneva'	*Vh3*	*Rvi3*	3	3	3	3	3	3	3	3
TSR18T13	*Vh4*	*Rvi4*	0	0	0	0	0	0	0	0
OR45T132	*Vm*	*Rvi5*	0	3	0	1	2	3	2	1
Freedom	*Vf, Vpoly*	*Rvi6*	0	0	0	0	0	0	0	0
Liberty	*Vf*	*Rvi6*	0	0	0	0	1	0	0	0
Reglindis	*Vf*	*Rvi6*	0	0	0	0	0	0	0	0
Remo	*Vf*	*Rvi6*	3	0	0	0	0	0	0	0
Topaz	*Vf*	*Rvi6*	0	0	0	0	0	2	0	0
Florina	*Vf, Vg*	*Rvi6*, *Rvi1*	0	0	0	0	0	0	0	0
Prima	*Vf, Vg*	*Rvi6*, *Rvi1*	0	0	0	0	0	0	0	0
Angold	*Vpoly*	-	3	3	1	1	2	1	1	1
Produkta	*Vpoly*	-	3	3	1	0	1	1	1	1

[1]Old name of resistance gene; [2]New name of the resistance gene, as it was proposed by Bus et al. (2011), according to the new nomenclature based on the GFG hypothesis

3.3. Marker analysis of disease resistance in old apple cultivars, and in genotypes/cultivars from the Hungarian breeding work

Resistance genes and QTLs encoding disease resistance against apple scab and fire blight were monitored in genotypes developed by the Hungarian breeding work, and in the 57 old apple cultivars, which were also studied on the field. The latter group of cultivars was also tested for powdery mildew resistance genes. We only found one allele associated with powdery mildew resistance, which is suggesting that the 'Búzával érő alma' cultivar might carry the *Pl-2* gene. This cultivar showed strong field resistance to powdery mildew, which confirms this data.

In many cases, the marker analysis of the Hungarian bred genotypes showed that the major resistance genes were inherited during the breeding work, as it was expected based on the phenotype (Table 3). For example, the *Rvi6* (syn. *Vf*) resistance gene originated from *Malus floribunda* is being carried by all of the new Hungarian resistant cultivars (‘Artemisz’, ‘Cordelia’, ‘Hesztia’ ‘Rosmerta’). As it was expected from the pedigree of ‘Artemisz’ we found that this cultivar carry the resistance QTLs (FLO5, FLO10, FB_MF) identified in ‘Florina’ and in *Malus floribunda* (table 4.). In several cases the results of the marker analysis gave unexpected explanation to the resistant phenotype of the new cultivars/genotypes. For example, ‘Hesztia’ was selected from open-pollinated seedlings, and yet it carries fire blight resistance alleles in three different linkage groups (LG5, LG7, and LG10).

Table 3. Allele sizes of scab resistance markers, in genotypes originating from the Hungarian breeding work.

Cultivar	*Rvi6*	*Rvi4*	*Rvi2=Rvi8*
	AL07	AD13	OPL19
Artemisz	570*/820	1100	433*/1200
Cordelia	570*/820	1100	1200
Hesztia	570*/820	1100	433*/1200
Rosmerta	570*/820	1100	1200
MA-14	570*/820	950*/1100	1200
MR-16 (B-216)	570*/820	1100	1200
MR-17 (B-203)	570*/820	1100	433*/1200
MV-04 (GFV-04)	820	1100	1200
Rodonit (MT-01)	820	1100	1200
Karneol (MT-11)	820	1100	433*/1200
Prima	570*/820	-	-
Malus pumila 'niedzwetzkyana'	-	950*/1100	-
Reka	-	-	433*/1200

*Allele size associated with resistance

Table 4. Allele sizes of FBF7, FLO5, FLO10 and FB_MF markers, in genotypes originating from the Hungarian breeding work.

Cultivar	FBF7		FLO5	FLO10			FB_MF	Phenotype (%)[1]
	AE10-375	GE-8019	Ch05e06	Ch02b07	CH01f12	CH02a10	Hi07f01-F	
Artemisz	375*	400	132/147	107/126*	152*/161	141/175*	206/219*	R (14,3)[2]
Cordelia	0	400	221	103/126*	148/161	139/145	206	MR (36,5)[2]
Hesztia	375*	400	132/139*	103/126*	161	145	206/208	R (12,3)[2]
Rosmerta	375*	400	221	105/126*	142/161	141/153	208/219*	MR (35)[2]
MA-14	0	400	145	103/108	148/161	153	206/219*	MR (35)[2]
MR-16	375*	400	145	108	152*	145/153	206/208	MR (31,6)[2]
MR-17	0	700*	134/139*	103/126*	152*/161	147/175*	206	R (5,4)[2]
MV-04	0	400	137/139*	105	152*/172	147/175*	201/204	(MS) 67,6[2]
Rodonit	0	400	132/150	105/111	145/161	141/145	206/219*	MR (50,7)[2]
Karneol	0	400	145/158	103/111	148/161	145/153	206/208	R (12,5)[2]
James Grieve	375*	700*	-	-	-	-	-	-
Florina	-	-	139*/150	126*	148/152*	153/175*	-	-
Malus floribunda	-	-	-	-	-	-	206/219*	-
Prima	-	-	-	-	-	-	-	R (24,3)[2]
Idared	-	-	-	-	-	-	-	S (86,1)[2]

*Allele size associated with resistance; [1]*R* - resistant, MR - moderately resistant, MS - moderately susceptible, S - susceptible; [2]Percentage of infected shoot length, after artificial infection (Tóth et al. 2012, Tóth et al. unpublished data)

The *Rvi6* (*Vf*) resistance gene originating from *Malus floribunda* could not be shown in any of the old cultivars by the marker analyses. However, the presence of *R* genes first identified in *Malus pumila* sel. R12740-7A were shown in many cases, which might be the result of the closer relation to this wild species (table 5). The presence of ephemeral scab resistance genes (*Rvi1*, *Rvi8*) was also typical. This data is interesting as these genes cannot be directly detected through phenotypic evaluations. The largest number of resistance alleles was found in 'Batul'. Based on our data this cultivar might carry the following scab resistance genes: *Rvi4*, *Rvi8*, and *Rvi1*.

Table 5. The most important scab resistance alleles detected in old apple cultivars of the Carpathian basin.

Cultivar	*Rvi4*		*Rvi2*		*Rvi8*	*Rvi1*	Phenotype (0-3)[1]
	AD13	CH05e03	CH05e03	OPL19	OPB18	Ch01d03	
Batul	950*	164*	164	433*/1200	628/799*	138/157*	R (0,45)
Bereczki Máté	950*/1100	162/164*	162/165	1200	628/799*	140/142	MR (0,54)
Budai Ignác	950*	164*/189	163/189	433*/1200	628	140/142	R (0,12)
Csíkos óriás halasi	1100/1300	161/172	161/172*	433*/1200	628/799*	136/142	R (0)
Izletes zöld	950*/1100	164*/189	164/189	433*/1200	628	142/157*	R (0)
Kis Ernő tábornok	1100	164*/184	164/184	433*/1200	628/799*	138/157*	MR (0,75)
Miskolci kormos	1100/1300	169/172	169/172*	433*/1200	0	136/157*	MR (1,04)
Pónyik	1100/1300	172/184	172*/184	433*/1200	628	136/142	R (0)
Sándor cár	1100/1300	161/172	161/172*	433*/1200	628/799*	136/144	R (0)
Vilmos renet	1100	162/172	162/172*	433*/1200	628	136	R (0,33)
Prima	-	-	-	-	-	-	-
Malus sieversii	950*/1100	159/164*	159/164	-	-	-	-
Reka	-	166/172	166/172*	433*/1200	628	-	-
Golden Delicious	-	-	-	-	-	154/157*	-

*Allele size associated with resistance; [1]R -resistant, MR - moderately resistant

By monitoring the known QTLs associated with fire blight resistance, the resistance QTL identified in the ornamental crabapple *Malus* × 'Evereste' could not be detected in any of the investigated cultivars. In contrast of this, the FBF7 QTL identified in 'Fiesta' explained the resistant phenotype of several cultivars (Table 6, e.g. 'Sikulai alma'). Interestingly, in three old cultivars ('Batul', 'Kéresi muskotály' and 'Szabadkai szercsika') the FB_MR5 resistance of *Malus robusta* was suggested by the detected allele size. This result is especially valuable, as the FB_MR5 QTL is the most important fire blight resistance factor to date, and it was shown to be carried by cultivated varieties for the first time at the international level.

Table 6. The most important alleles in the old cultivars of the Carpathian basin, linked to fire blight resistance QTLs

Cultivar	FBF7		FB_MR5		FB_E	Phenotype (%)[1]
	AE10-375	GE-8019	FEM47	FEM19	ChFbE06	
Batul	0	220/700	191/193/209*/216	157	237	21.45
Cserepanya	375*	700/400*	191/193/216	134/157	237	55,5
Kéresi muskotály	0	220	191/192/209*/216	132/155	237	14,55
Pónyik	0	220	193/216	132/155	225/237	7,2
Sikulai alma	375*	220/400*	191/193/216	132/157	233/237	7,2
Simonffy piros	375*	220/400*	191/216	157/170	233/237	62,25
Szabadkai szercsika	375*	220	191/192/193/209*/216	132/157	233/237	6,5
Kisasszony	375*	220/700	191/193/216	132	237	86,5
Tafota	375*	220/400*	191/193/200/216	124/134	233/237	50,75
Vajki alma	375*	220/400*	191/193/200/216	124/134	233/237	41,2
James Grieve	375*	220/400*	-	-	-	-
Malus × robusta 5	-	-	193/209*/216	132/150*	-	-
Malus × 'Evereste'	-	-	-	-	273*[2]	-
Idared	-	-	-	-	-	89,7

*Allele size associated with resistance; [1] Percentage of infected shoot length, after artificial infection according to the data of Tóth et al. (2013); [2]literature data (Parravicini et al. 2011)

3.4. Sequence analysis of the OPL19 amplicon

The marker analysis revealed the extremely high allele frequency of the OPL19 marker. This problem should be clarified also for carrying out the future analyses correctly. Comparing the OPL19 sequence of ‘Batul’ with other sequences uploaded to the GeneBank, we found that the susceptible cultivars have a different amplicon which is similar in its size, but differs in its sequence. This might lead us to false assumptions concerning the presence of the linked resistance gene. However, our results confirmed that Batul carries the Rvi8 resistance gene.

3.5. New scientific results

1. Five new gene sources were selected on the basis of my field study on apple scab and powdery mildew and the previous *Erwinia* research of the Dept.: ‘Angyal Dezső’, ‘Cigány alma’, ‘Damjanich’, ‘Dániel féle renet’, ‘Szemes alma’.

2. Evaluating the susceptibility of scab race indicator cultivars on the field for eight years, we confirmed that the Hungarian population of the *Venturia inaequalis* pathogen has changed. Based on this, the presence of races 1; 3 and 5 could clearly be proved by the field study. Race 4 is not present, while the presence of *Venturia* races 2; 6 and 7 is still questionable due to the inadequate quantity, and quality of data.

3. The resistance genes of *Vr* resistance (*Rvi2* and *Rvi4*), and the ephemeral genes *Rvi1* and *Rvi8*, were detected in several old apple cultivars from the Carpathian basin.

4. We detected the FB_MR5 QTL in cultivated varieties for the first time at the international level.

5. We confirmed that the pyramidation and combination of the genes for achieving apple scab and fire blight resistance in the six new cultivars and cultivar candidates developed by the Dept. of Pomology (‘Artemisz’, ‘Hesztia’, ‘Rosmerta’, MR-16, MR-17 and MA-14) was successful.

4. Conclusions and recommendations

Several old apple cultivars were selected, which have strong disease resistance based on the phenotypic evaluations and the results of the marker analyses. On the one hand, these cultivars might play an important role in the future breeding works; on the other hand, due to their disease resistance they might be excellent cultivars for ecological orchards. However, other cultivar characteristics can be decisive in the practice, thus it would be necessary to study these cultivars further on the field for many years to determine their potential in the apple production. In the case of some cultivars (e.g. 'Batul'), many variants are known of the "base" cultivar. In these cases, collecting further variants may open possibilities for breeders and growers, not mentioning the cultural and rural development concerns of the topic.

According to the Hungarian occurrences of the *Venturia inaequalis* races, resistant cultivars carrying the *Rvi2* and *Rvi4* genes should be involved to the breeding more often. However, more accurate markers would be needed than OPL19 to monitor *Rvi2*. It would be necessary to place the internationally used indicator cultivar set to several places of the country, and monitor the occurrences of the further races.

Our results are suggesting that the combination of major genes results in stronger and more stable resistance. So it would be important to use molecular markers in the future breeding works. If a cultivar carries more than one major resistant gene, it can be detected clearly and accurately by this method. In many cases the marker analysis gave the same results concerning the inheritance of major resistance genes as we expected it based on the phenotype. The results are proving that in the newly bred Hungarian cultivars many resistance genes are pyramided, which results in more stable resistance; furthermore they draw attention to the efficiency of marker analyses in the breeding.

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