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Scab Resistance in some Apples Genotypes from the UASVM Collection Cluj-Napoca

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RESEARCH ARTICLE

Abstract
Apple is one of the most consumed fruits in the world and as well one of the most important crops in temperate areas. Apple scab, caused by *Venturia inaequalis* is damaging diseases that affects apple species and causes up to 70% yield losses depending of cultivar's resistance. In order to control this disease, costly chemical pesticides are necessary, which increase the pollution of environment by their toxicity. This study is focused on checking the behaviour of certain apple varieties from the pomological collection at UASVM Cluj-Napoca against apple scab infection. Nine apple cultivars ('Fuji Kiku', 'Red Cap', 'Lena', 'Katja', 'Gala Mitchgla', 'Fiesta', 'Yellow', 'Pinova' and 'Reinette du Canada'), were analysed in terms of behaviour to scab attack on leaves and fruits. Visual observations were completed by molecular analyses for the potential presence of the *Vf* gene in some cases. Combining results from visual observation and molecular assays, the 'Lena' and 'Pinova' cultivars were noted as resistant to scab in our field trial conditions. This indicates a good opportunity to bring them into apple breeding programs for resistance to apple scab.

Keywords: apple, scab, symptoms, behavior, resistance, MAS

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INTRODUCTION

According to the most recent FAOSTAT data (2021), the apple species (*Malus domestica* Borkh.) ranks second in terms of both area (53,820 ha) and production (593,700 t) after plum in Romania. The apple is affected by some diseases with a strong economic impact. One of these diseases is apple scab, caused by the fungus *Venturia inaequalis* (Cke) Wint, considered one of the most devastating diseases of apple trees, which can lead to production losses of up to 70%, depending on the variety's resistance (Militaru et al. 2020). The major drawback for an efficient control of apple scab to obtain good quality of fruit, is the large number of treatments being required (approximately 20-30 treatments/season) in the orchard (Boyraz et al., 2005; Soriano et al., 2009). For this reason, it is considered one of the most treated crops in the world (Aćimović, 2020). This brings about negative effects, on one hand on the environment and consequently on human health, and on the other hand, unfortunately, the pathogen has developed resistance to many categories of fungicides currently on the market. Using cultivars with resistance to apple scab is one of the most effective methods to limit the spread of the fungus and, consequently, to reduce the number of treatments. Apple resistance to scab can be of two types: monogenic and polygenic (Durel et al., 2003). The polygenic resistance is more stable and durable than the monogenic one. However, due to difficulties in its integral transferring to

progenies by hybridization is often avoided in breeding work. In apple breeding programs, the most commonly used is the monogenic resistance to scab based on *Rvi6* (*Vf*) gene, identified in a wild cultivar *Malus floribunda* clone 821.

The *Rvi6* gene has been widely used as a source of resistance to apple scab in most commercial varieties (Crosby et al., 1992). However, it is known that *Vf*-type resistance provides limited protection. Race 6 overcomes this type of resistance, although *Malus floribunda* clone 821 remains an effective source of resistance on its own (Parisi et al., 1993; Masny, 2017). According to recent studies in the field, symptoms of apple scab have been identified on a number of cultivars containing the *Rvi6* gene (Militaru et al., 2019; Roşu-Mareş et al., 2022).

The aim of this study was to check the behavior of certain apple cultivars grown in pomological collection of USAVM Cluj-Napoca, against apple scab infection.

MATERIALS AND METHODS

The experiment consisted in evaluation of the behavior of various apple tree cultivars to attacks of apple scab on fruits and leaves. Additionally, molecular methods were used to identify the *Rvi6* (*Vf*) resistance gene. The study material consisted of nine apple cultivars (Table 1), which exhibited varying degrees of sensitivity to scab attacks.

Table 1. The apple cultivars included in the study and their genitors

Cultivar	Genitors
'Fuji Kiku'	♀ Red delicious x ♂ Virginia Ralls Janet ('Rawls Jennet)'
'Red Cap' sin. Valtod	natural mutants of the Red delicious
'Lena'	♀ Rajka x ♂ Topaz
'Katja'	♀ James Grieve x ♂ Worcester Pearmain
'Gala Mitchgla'	♀ Kidd's Orange Red x ♂ Golden delicious
'Fiesta' sin. Red Pippin	♀ Cox's Orange Pippin x ♂ Idared
'Yellow'	♀ Golden delicious x ♂ Senshu
'Pinova'	♀ Clivia x ♂ Golden delicious
'Reinette du Canada'	unknown

Field visual observations

The apple collection was treated with at least four sprays against apple scab using the following substances: captan 1,250 g/ha, tebuconazole 325 g/ha, dithianone 312,5 g/ha and potassium phosphonate 1,402.5 g/ha combined with insecticides against pests. Visual observations of scab symptoms were performed on a sample of 300 leaves and 300 fruits from each variety, randomly harvested from the orchard in the first decade of September 2023. In order to determine the damage degree of scab on the studied varieties, the frequency and intensity were calculated. Thus, the frequency (F) was calculated as a percentage of infected organs from the total organs observed and the intensity (I) was assessed using the 7-level scale (Table 2) according to specific phytopathological methodologies.

Table 2. The scale used for scab observation

Level of intensity	Percentage of foliar surface affected by scab symptoms
0	0
1	0.1-5
2	5-10
3	11-25
4	26-50
5	51-75
6	>75
I = (nx5 + nx10 + nx25 + nx50 + nx75 + nx100)/N N = number of organs with symptoms from each level of intensity N = total number of organs with symptoms	

Note: The damage degree was calculated according to the formula: DD=(FxI)/100

Statistical analysis

Statistical analysis was performed for: (a) differences between scab damage degrees on leaves for all cultivars tested; (b) differences between scab damage degrees on fruits for all cultivars tested. The data was analyzed using the XLSTAT - Addinsoft software (Addinsoft, 2022), which utilize the MS Office Excel platform. All data collected from the field was arranged in completely randomized blocks, and after the XLSTAT program was used to the analysis of variance (ANOVA – Fisher, 1925). Afterwards, the Duncan's Multiple Rage Test was used to analyze the differences degrees of significance between the different variants (Duncan, 1955) at $p<0.0001$.

Molecular analysis

To identify a gene that confers resistance to apple scab, specifically the *Rvi6* (*Vf*) gene, molecular analyses were conducted using three sets of specific primers: one dominant pair (AM19) and two co-dominant primer pairs (AL07 and VfC) that allow distinguishing between homozygous and heterozygous genotypes. The molecular analyses followed the steps of DNA extraction, amplification of the extracted DNA and migration of PCR products on agarose gel. DNA isolation was performed using the Invisorb Spin Plant Mini Kit (Bioline), following the manufacturer's provided protocol, resulting in a final quantity of 50 µl of pure DNA. For amplification, the MyTag Red Mix (Bioline) was used, resulting in a final quantity of 25 µl per tube for each sample. The amplification of the DNA took place in the Mastercycler gradient (Eppendorf). The thermal cycling schemes used for AL07 and AM19 primer pairs were according to Khajuria et al., 2014, and Tartarini et al., 1999, and for VfC primer pair, according to Afumian et al., 2004. The migration of amplified products was carried out onto 1.5% agarose gel for 50 minutes using RedSafe Nucleic Acid Staining Solution. Bands were visualized using the Quantity One 1-D Analysis Software system under UV light.

RESULTS AND DISCUSSIONS

Scab symptoms on leaves and fruits

By analyzing the nine apple cultivars in terms of scab symptoms on leaves and fruits was observed that they were attacked by *Venturia inaequalis* to varying degrees.

Figure 1 illustrates the parameters assessed by visual observation in the field on the leaves at the end of summer, 2023. Excepting 'Lena', all the other eight cultivars were damaged by *Venturia inaequalis*. 'Fuji Kiku' had the highest frequency of scab symptoms on leaves, and also a high intensity level of scab, which expressed the highest damage degree. Considering the low number of treatments against scab performed combined with favorable climatic conditions for disease development, we can assume that the results are suitable to assess the level of susceptibility of the monitored cultivars.

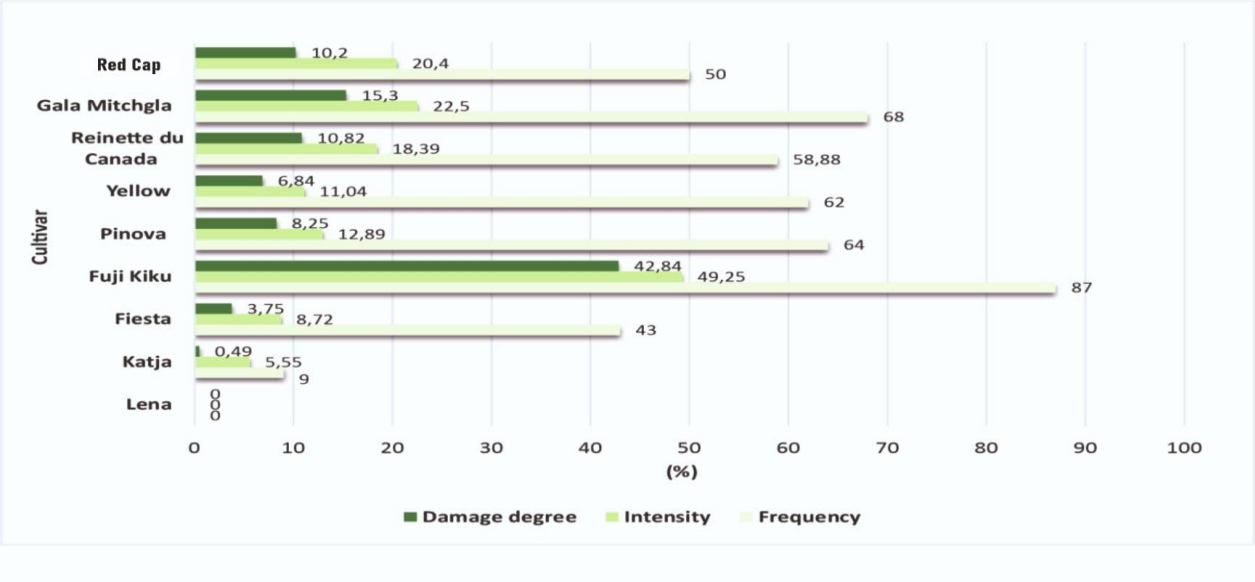

Figure 1. Damage degree/intensity/frequency of scab symptoms on the leaves.

The visual observation of leaves performed in the field trial revealed that the 'Lena', had no scab lesions suggesting that the resistance given by the *Rvi6* gene remains effective in our field conditions. In spite of possessing the *Rvi5*, *Rvi6*, and *Rvi11* genes, 'Pinova' (Podwyszyńska et. al 2021) showed symptoms of scab on the leaves, but

only 8.25% of the foliar surface being damaged. The cultivar 'Fuji Kiku', known to be highly susceptible to scab, was the most affected by *Venturia inaequalis* infections, 42.84% of the foliage was damaged. All the other cultivars had damage degrees between 0.49% ('Katja') and 15.30% ('Gala Mitchgla'), confirming the lack of resistance to natural infections with *Venturia inaequalis.*

The parameters assessed by visual observation in the field on the fruits at the end of summer 2023, suggested that all cultivars presented symptoms of scab on the fruit. Thus, all the inspected fruits were damaged by one or multiple scab lesions. The most susceptible cultivar to apple scab, in terms of fruit damage degree was 'Gala Mitchgla' (54.89%). Although the next highest frequency belongs to cultivar 'Yellow' (89.28%), the second most affected was 'Fuji Kiku' whose damage degree was 17.7% as a result of high intensity of symptoms (Figure 2).

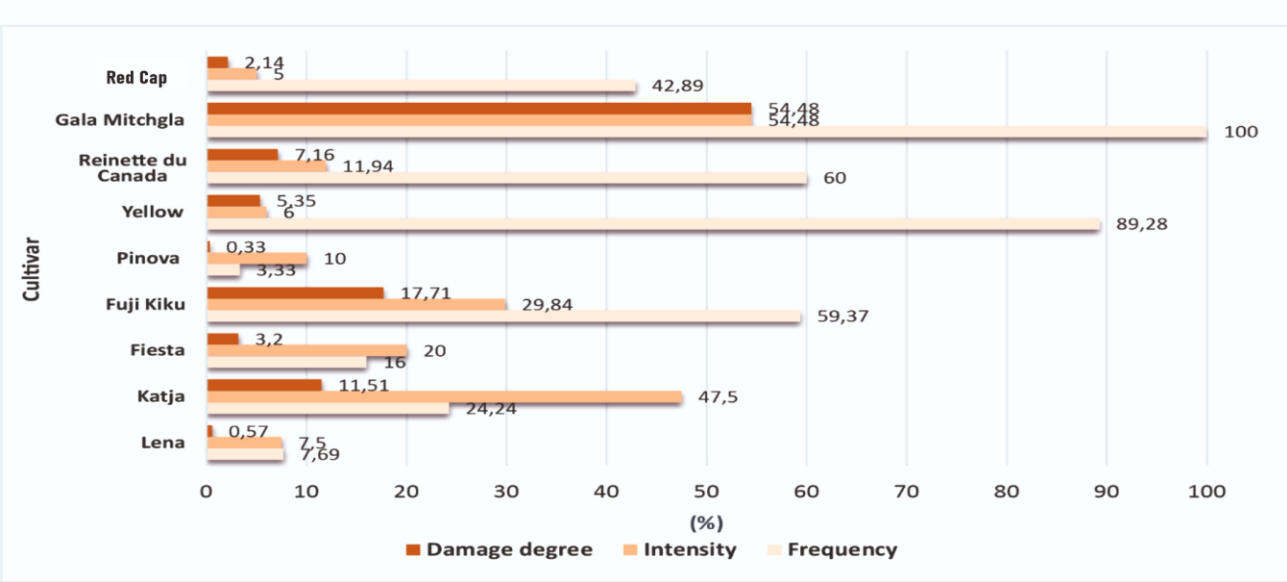

Figure 2. Damage degree, intensity and frequency of scab symptoms on fruits

The fewest symptoms of scab on fruits were recorded on 'Pinova' (0,33%) and 'Lena' (0,57%) cultivars, which contain in their genome resistance gene. The nine cultivars showed different symptoms of scab attack on fruits. It appears in the form of irregular round spots of gray-olive color, where the tissues become corky and crack (Figure 3). Often, the fruits become severely deformed and fall off.

Figure 3. The presence of scab symptoms on the fruits of the nine apple cultivars studied

Statistical analysis

Cultivars 'Lena' and 'Katja' showed no significant differences between their values of damage degree on leaves, but significat differences between the damage degree on fruits, in case of 'Katja'. The same applies to cultivars 'Reinette
du Canada' and 'Red Cap', there are no significant differences between the average damage degree on leaves, but significant differences between the average damage degree on fruits (Tabel 3). All the other cultivars significantly differ from each other regarding the average damage degree on leaves.

The values presented in table 3 are averages of scab damage degree on the fruits of the apple cultivars from the trial. The cultivars showing no significant differences between the average damage degree on fruits are 'Pinova' and 'Lena'. Both of them performed well under the climatic conditions of the 2023, the value of the damage degree on fruits was small in both cases: 0.32% (Pinova) and 0.56% (Lena). All the others cultivars fruits were significantly more damaged by scab and significantly different from each other.

Table 3. Values of damage degree on leaves and fruits (mean ± S.E. in %) for all apple cultivars studied

Leaves		Fruits	
Cultivar	**Damage degree**	**Cultivar**	**Damage degree**
Fuji Kiku	42.837 ± 0.50 ^{g}	**Gala Mitchgla**	54.483 ± 2.14 ^{h}
Gala Mitchgla	15.303 ± 0.35 ^{f}	**Fuji Kiku**	17.710 ± 4.27 ^{g}
Reinette du Canada	10.817 ± 0.12 ^{e}	**Katja**	11.507 ± 2.11 ^{f}
Red Cap	10.200 ± 0.41 ^{e}	**Reinette du Canada**	7.157 ± 2.16 ^{e}
Pinova	8.250 ± 0.15 ^{d}	**Yellow**	5.347 ± 1.52 ^{d}
Yellow	6.843 ± 0.46 ^{c}	**Fiesta**	3.203 ± 0.90 ^{c}
Fiesta	3.750 ± 0.04 ^{b}	**Red cap**	2.140 ± 1.00 ^{b}
Katja	0.487 ± 0.08 ^{a}	**Lena**	0.567 ± 0.35 ^{a}
Lena	0.000 ± 0.00 ^{a}	**Pinova**	0.327 ± 0.12 ^{a}
Pr > F(Model)	0.001		
Significant	Yes		

Note: The values presented in the table are averages of scab damage degree on the leaves for every apple cultivar. Averages followed by different letters indicate differences at $p < 0.0001$ according to Duncan`s Multiple Range Test.

Following statistical processing (XLSTAT) regarding fruit scab symptoms, 'Lena' and 'Pinova' cvs. behaved similarly, despite the statistical differences obtained in the case of leaf attack.

Molecular analyses

Following the molecular assays, the *Rvi6* (*Vf*) gene was amplified in two out of nine studied apple cultivars, respectively 'Lena' and 'Pinova'cultivars(Figure 4, Table 4), by using three specific primers pairs: AL07 at 570 bp (*Vf*) and 823 bp (*vf*); AM19 at 526 bp (*Vf*) and VfC at 286 bp (*Vf*), as well as 484 bp and 646 bp (*vf*).

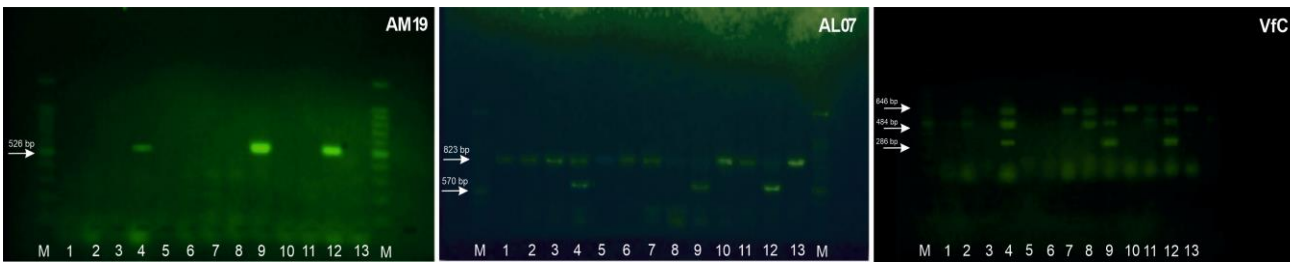

Figure 4. Electrophoresis profile of genotype identified on nine apple cultivars
M – marker, 1 – 'Golden delicious' (C-), 2 - 'Fuji Kiku', 3 - 'Red Cap', 4 - 'Lena', 5 -'Katja', 6 – 'Gala Mitchgla', 7 – 'Fiesta', 8 – 'Yellow', 9 – 'Pinova', 10 – 'Reinette du Canada', 11 – 'Idared' (C-), 12 – 'Florina' (C+), 13 – 'Auriu de Bistrița' (C-)

Table 4. The results of molecular assays of apple cultivars regarding the resistance to apple scab

Apple cultivar	Primers pair sets		
	AL07	AM19	VfC
	Vf	*Vf*	*Vf*
'Golden delicious' (negative control)	-	-	-
'Fuji Kiku'	-	-	-
'Red Cap' sin. Valtod	-	-	-
'Lena'	+	+	+
'Katja'	-	-	-
'Gala Mitchgla'	-	-	-
'Fiesta' sin. Red Pippin	-	-	-
'Yellow'	-	-	-
'Pinova'	+	+	+
'Reinette du Canada'	-	-	-
'Idared' (negative control)	-	-	-
'Florina' (positive control)	+	+	+
'Auriu de Bistrita' (negative control)	-	-	-

+/- = the presence/absence of the respective amplified allelic fragments linked to *Rvi6* resistance gene

Similar results regarding susceptibility/potential resistance to scab in the tested apple cultivars in the current study have also been reported in other previous experiments (Belete and Boyraz, 2017; Arici et al., 2014 for the 'Yellow' cultivar, Didelot et al., 2016 for the 'Gala Mitchgla' cultivar, and Patzak et al., 2011 for the 'Reinette du Canada' cultivar).

CONCLUSIONS

The behavior of nine apple varieties from the UASVM Cluj-Napoca collection to scab attack, under minimal phytosanitary treatment conditions, highlighted a good protection against scab of 'Lena' and 'Pinova' cultivars that were only slightly affected due to the presence resistance gene/-s in their genome.

Author Contributions: B.G.M. performed the molecular analyses, collected the data and wrote the paper; Z.I. contributed data or analysis tools and wrote the paper; R.-M.S.D. and M.C. conceived and designed the analysis; wrote the paper; Z.L. conceived and designed the analysis; B.O, A.A., C.M.I collected the data.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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