

## MARKER ASSISTED SELECTION FOR RESPONSE ATTACK OF *VENTURIA INAEQUALIS* IN DIFFERENT APPLE GENOTYPES

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**Abstract.** Apple scab caused by *Venturia inaequalis*, is an important disease, spread worldwide, having a major negative impact in apple production. In this study, there were analyzed four apple cultivars (Liberty, Florina, Starkrimson, Golden Spur), used as genitors in a diallel cross, and their seedlings. In the field, plants selection was done after presence or absence of the scab on leaves, and afterwards, phenotypically selection was completed by marker assisted selection. The molecular analysis conducted with the markers AL-07, AM-19 and U1<sub>400</sub>, detects the presence of the *Vf* gene, introgressed from *Malus floribunda* 821, which confers resistance to the pathogen, in the parental cultivars Liberty and Florina, and in all the F<sub>1</sub> hybrids belonging these two genitors. Starkrimson and Golden Spur were susceptible scab parents with recessive homozygous genotype (*vf/vf*), while Liberty and Florina were identified as heterozygous scab resistant parents with genotype (*Vf/vf*). From the 60 apple seedlings analyzed, grouped in six combinations after their genitors (in each combinations were selected ten hybrids, among them five resistant and five susceptible to apple scab attack) 28 were resistant and heterozygous for *Vf* gene (*Vf/vf*) and 32 were susceptible to scab with recessive homozygous genotypes (*vf/vf*), being obtained almost Mendelian ratio for response to the pathogen, 50% resistant: 50% susceptible. Seedlings belonging to Starkrimson x Golden Spur were only recessive homozygous genotype (*vf/vf*), and in combination resistant x resistant (Liberty x Florina) were identified susceptible and resistant offspring, but at last, only with heterozygous for *Vf* gene (*Vf/vf*) and no one with dominant homozygous genotypes (*Vf/Vf*).

**Key words:** apple, scab resistance, molecular markers, *Vf* gene, MAS

### INTRODUCTION

Apples are the most important fruit species in the temperate zone, where is widely spread and cultivated, so it has a considerably economic role. The fruits contain dietetically important substances, thereby they have an important role in human nutrition. Unfortunately, most of the commercial cultivars are susceptible to the apple scab, caused by fungus *Venturia inaequalis*, which attacks both leaves and fruits, reducing yield and quality.

Apple scab is currently controlled by frequent application of fungicides, in consequence increasing production costs as well as rising ecological and health concerns (Tartarini et al., 1999). For these reasons, apple breeding programs have been orientated to create new scab resistant cultivars. In Romania, especially in Transylvania, climatic conditions are extremely favourable for disease development and spread, therefore an important desideratum in apple breeding is to obtain new apple commercial cultivars resistance to the pathogen (Sestras, 2004).

In *Malus floribunda* Sieb. clone 821 was identified a major dominant gene (Hough et al., 1953), called *Vf* gene (*V*, from *Venturia*, named of the pathogen, and *f* from *M. floribunda*), which gives resistance to *Venturia inaequalis* pathogen. In cultivated apple varieties this resistance has been acquired by introgression of the *Vf* resistance gene.

This resistance has been utilized in apple-breeding programs through the world for more than 40 years and has been incorporated into a substantial number of apple cultivars (Crosby et al., 1992). Apple breeders are on a continuous search for new apple scab resistance genes because the *Vf* resistance has been overcome by *V. inaequalis* races 6 and 7 (Parisi et al., 1993; Bénaouf and Parisi, 2000). At least six major independent genes conferring resistance to *V. inaequalis* have been identified in apple germplasm: *Vf* from *Malus floribunda* 821, *Vm* from *M. micromalus*, *Vb* from *M. baccata* Hansen's no. 2, *Vbj* from *M. baccata* var. *jackii*, *Vr* from *M. pumila* R12740-7A, and *Va* from the Antonovka PI 172623 (Xu and Korban, 2000). There are several most important scab resistance gene sources for breeding resistance to apple scab which are used either alone or in combination to prevent, or at least to slow down, resistance breakdown (Vinatzer et al., 2004), but only the *Vf* gene has been widely introgressed into susceptible commercial apple cultivars (Korban, 1998).

The identification of molecular markers linked to the *Vf* gene is useful in marker-assisted selection (King et al., 1999; Tartarini et al., 2000) and the AFLP technique has proven to be a powerful tool for identifying tightly linked markers which are indispensable to marker-assisted selection (Xu and Korban, 2000).

## MATERIAL AND METHOD

Biological material was represented by four apple cultivars (Starkrimson, Golden Spur, Liberty and Florina) and their seedlings (F<sub>1</sub> hybrids), obtained by a diallel cross after Griffing method (1956). Hybridizations were made at Fruit Research Station, Cluj-Napoca, Romania, where were studied the following combinations:

- 9811 = Liberty x Florina;
- 9812 = Starkrimson x Golden Spur;
- 9813 = Starkrimson x Florina;
- 9814 = Starkrimson x Liberty;
- 9817 = Golden Spur x Florina;
- 9818 = Golden Spur x Liberty.

The F<sub>1</sub> hybrids originated from parents with a different response at scab attack: susceptible (Starkrimson and Golden Spur); resistance (Liberty and Florina) these having a scab genetic resistance, carrying by *Vf* gene.

From each combination, with more than 100 seedlings, were selected ten hybrids: five resistant at scab attack on leaves and five susceptible at scab attack on leaves (64 samples, among them, four being represented by genitors and 60 apple seedlings, grouped in six combinations – Table 7).

Leaves from each plant were collected in plastic bags, brought immediately to the laboratory and stored at -80°C.

DNA extraction was performed from leaf material according to Lodhi's et al. (1994) protocol. PCR reactions were carried out in 25 µl volumes containing 5X Green GoTaq Flexi reaction buffer, 1.5 mM MgCl<sub>2</sub>, 200µM of each dNTP, 0.2 µM of each primer:

- AL07F-5'-TGGAAGAGAGATCCAGAAAGTG-3' and
- AL07R-5'-CATCCCTCCACAAATGCC-3';
- AM19-F-5'-CGTAGAACGGAATTTGACAGTG-3' and

AM19-R-5'GACAAAGGGCTTAAGTGCTCC-3')

U1<sub>400</sub> F 5'-GTAAAGCAAGCACTTCAACG-3' and

U1<sub>400</sub> R 5'-GTAAAATAGATGTGTGGGTAGC-3' and 0.6U of GoTaq polymerase.

The amplification was performed on a Mastercycler Gradient (Eppendorf) programmable thermal cycle. Cycling parameters for the AL-07 and AM-19 primers were set as described by Tartarini et al. (1999) as follows: one cycle of denaturation at 94°C for 2 min and 30 s, annealing at 60°C for 1 min, extension at 72°C for 2 min, and 35 cycles of 30 s denaturation at 94°C, 1 min annealing at 60°C, and 2 min extension at 72°C finalized by a final extension step 10 min at 72°C.

Cycling parameters for the U1<sub>400</sub> primer set as described by Hemmat et al. (1998) as follows: 2 min at 94°C, 2 min at 69°C, and 2 min at 72°C. The cycle was repeated each time 1 min at 94°C with a reduction of 1°C for annealing temperature per cycle. The cycle was repeated 30 times when annealing temperature reached 62°C. A final extension cycle of 10 min at 72°C was applied.

PCR products were run in 2% agarose gel, 1 hour at 90V in TAE (Sambrook et al., 1989) buffer and visualized by ethidium bromide (0.5 µg/ml) staining. 100 bp Step Ladder was used as a size marker. Images were acquired using an ALPHA IMAGE 2200 system under UV light.

## RESULTS AND DISCUSSIONS

After the DNA extraction from four apple cultivars: Starkrimson, Golden Spur, Florina and Liberty and sixty hybrids F<sub>1</sub> (ten seedlings: five resistant and five sensitive to apple scab on leaves) obtained by combinations between Liberty and Florina, Starkrimson and Golden Spur, Starkrimson and Florina, Starkrimson and Liberty, Golden Spur and Florina, Golden Spur and Liberty cultivars, it was obtained different concentration.

In the combination 9811 Liberty x Florina (Table 1), the average of DNA concentration, respectively DNA purity for susceptible hybrids is 460.2 and 1.56; for resistant hybrids the value is 634.0 and 1.56.

Table 1

The values of DNA concentration and purity for combination 9811 Liberty x Florina

Cultivar/Genotype	DNA concentration ng/µl	DNA purity
Liberty	956.0	1.94
Florina	471.5	1.64
Hybrid 1 susceptible to scab attack on leaves	426.9	1.42
Hybrid 2 susceptible to scab attack on leaves	628.9	1.90
Hybrid 3 susceptible to scab attack on leaves	429.8	1.40
Hybrid 4 susceptible to scab attack on leaves	263.3	1.25
Hybrid 5 susceptible to scab attack on leaves	552.0	1.84
Hybrid 6 resistant to scab attack on leaves	588.2	1.33
Hybrid 7 resistant to scab attack on leaves	440.9	1.57
Hybrid 8 resistant to scab attack on leaves	721.4	1.95
Hybrid 9 resistant to scab attack on leaves	555.6	1.14
Hybrid 10 resistant to scab attack on leaves	863.8	1.83

In the combination 9812 Starkrimson x Golden Spur (Table 2), the average of DNA concentration, respectively DNA purity for susceptible hybrids is 416.76 and 1.54; for resistant hybrids the value is 410.0 and 1.34.

Table 2

The values of DNA concentration and purity for combination 9812 Starkrimson x Golden Spur

Cultivar/Genotype	DNA concentration ng/μl	DNA purity
Starkrimson	710.9	1.67
Golden Spur	411.1	2.03
Hybrid 1 susceptible to scab attack on leaves	164.4	1.22
Hybrid 2 susceptible to scab attack on leaves	338.7	1.33
Hybrid 3 susceptible to scab attack on leaves	432.5	1.87
Hybrid 4 susceptible to scab attack on leaves	780.6	1.58
Hybrid 5 susceptible to scab attack on leaves	367.6	1.70
Hybrid 6 resistant to scab attack on leaves	325.2	1.51
Hybrid 7 resistant to scab attack on leaves	497.2	1.46
Hybrid 8 resistant to scab attack on leaves	244.6	1.34
Hybrid 9 resistant to scab attack on leaves	572.1	1.13
Hybrid 10 resistant to scab attack on leaves	411.1	1.28

In the combination 9813 Starkrimson x Florina (Table 3) the average of DNA concentration, respectively DNA purity for susceptible hybrids is 864.9 and 1.78; for resistant hybrids the value is 995.5 and 1.76.

Table 3

The values of DNA concentration and purity for combination 9813 Starkrimson x Florina

Cultivar/Genotype	DNA concentration ng/μl	DNA purity
Starkrimson	710.9	1.67
Florina	471.5	1.64
Hybrid 1 susceptible to scab attack on leaves	51.1	1.43
Hybrid 2 susceptible to scab attack on leaves	2857.9	1.82
Hybrid 3 susceptible to scab attack on leaves	1394.8	1.84
Hybrid 4 susceptible to scab attack on leaves	1399.6	2.02
Hybrid 5 susceptible to scab attack on leaves	20.7	1.80
Hybrid 6 resistant to scab attack on leaves	1606.9	2.00
Hybrid 7 resistant to scab attack on leaves	1802.7	1.65
Hybrid 8 resistant to scab attack on leaves	501.8	1.67
Hybrid 9 resistant to scab attack on leaves	15.4	1.81
Hybrid 10 resistant to scab attack on leaves	1050.6	1.66

In the combination 9814 Starkrimson x Liberty (Table 4) the average of DNA concentration and DNA purity for susceptible hybrids is 1103.5 and 1.76; for resistant hybrids the value is 802.5 and 1.63.

Table 4

The values of DNA concentration and purity for combination 9814 Starkrimson x Liberty

Cultivar/Genotype	DNA concentration ng/μl	DNA purity
Starkrimson	710.9	1.67
Liberty	956.0	1.94
Hybrid 1 susceptible to scab attack on leaves	2111.2	1.75
Hybrid 2 susceptible to scab attack on leaves	1105.8	1.63
Hybrid 3 susceptible to scab attack on leaves	764.5	1.70
Hybrid 4 susceptible to scab attack on leaves	621.7	1.84
Hybrid 5 susceptible to scab attack on leaves	914.5	1.90
Hybrid 6 resistant to scab attack on leaves	1372.1	1.96
Hybrid 7 resistant to scab attack on leaves	487.8	1.73
Hybrid 8 resistant to scab attack on leaves	726.4	1.54
Hybrid 9 resistant to scab attack on leaves	567.7	1.5
Hybrid 10 resistant to scab attack on leaves	858.5	1.43

In the combination 9817 Golden Spur x Florina (Table 5) the average of DNA concentration and DNA purity for susceptible hybrids is 838.4 and 1.86; for resistant hybrids the value is 674.7 and 1.95.

Table 5

The values of DNA concentration and purity for combination 9817 Golden Spur x Florina

Cultivar/Genotype	DNA concentration ng/μl	DNA purity
Golden Spur	411.1	2.03
Florina	471.5	1.64
Hybrid 1 susceptible to scab attack on leaves	497.0	1.51
Hybrid 2 susceptible to scab attack on leaves	903.3	2.01
Hybrid 3 susceptible to scab attack on leaves	706.2	1.95
Hybrid 4 susceptible to scab attack on leaves	1038.1	1.93
Hybrid 5 susceptible to scab attack on leaves	1047.5	1.88
Hybrid 6 resistant to scab attack on leaves	940.7	1.72
Hybrid 7 resistant to scab attack on leaves	458.0	2.03
Hybrid 8 resistant to scab attack on leaves	697.0	2.03
Hybrid 9 resistant to scab attack on leaves	604.9	1.98
Hybrid 10 resistant to scab attack on leaves	672.7	2.01

In the combination 9818 Golden Spur x Liberty (Table 6) the average of DNA concentration and DNA purity for susceptible hybrids is 634.2 and 1.90; for resistant hybrids the value is 552.6 and 1.96.

Table 6

The values of DNA concentration and purity for combination 9818 Golden Spur x Liberty

Cultivar/Genotype	DNA concentration ng/μl	DNA purity
Golden Spur	411.1	2.03
Liberty	956.0	1.94
Hybrid 1 susceptible to scab attack on leaves	417.5	2.02
Hybrid 2 susceptible to scab attack on leaves	750.0	1.79
Hybrid 3 susceptible to scab attack on leaves	717.5	1.82
Hybrid 4 susceptible to scab attack on leaves	589.7	1.95
Hybrid 5 susceptible to scab attack on leaves	696.3	1.92
Hybrid 6 resistant to scab attack on leaves	661.4	1.96
Hybrid 7 resistant to scab attack on leaves	512.2	1.95
Hybrid 8 resistant to scab attack on leaves	477.5	1.99
Hybrid 9 resistant to scab attack on leaves	348.2	2.00
Hybrid 10 resistant to scab attack on leaves	763.6	1.91

DNA concentration was measured through the spectofotometric method with the Eppendorf Biophotometer. Al-07 is a codominant primer, while AM-19 and U1<sub>400</sub> are dominant primers; all of them are specific primers for the *Vf* gene.

Specific primer AL-07 produced a clear length polymorphism in different genotypes with two products of 823 and 570 bp, AM-19 primer produced one products of 526 bp and U1<sub>400</sub> primer produced one products of 338 bp.

For the combination 9811 Liberty x Florina results of the molecular analysis with the primers AL-07, AM-19 and U1<sub>400</sub> are presented in Figure 1, 2 and 3.

In figure 1, the AL-07 570 bp (*Vf*) and 823 bp (*vf*) alleles are in coupling with, respectively, the resistant and susceptible allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Liberty heterozygous scab resistant parent with genotype (*Vf**vf*); and in columns 2 is represented Florina heterozygous scab resistant parent

with genotype (*Vfvf*). Columns 3, 4, 5 represented seedlings of this combination (9811 Liberty x Florina), with recessive homozygous genotypes (*vfvf*). Columns 6, 7, 8, 9, 10, 11, are represented by the seedlings of the same combination with heterozygous genotype (*Vfvf*). L is a 100 bp ladder molecular weight marker and C is negative control.

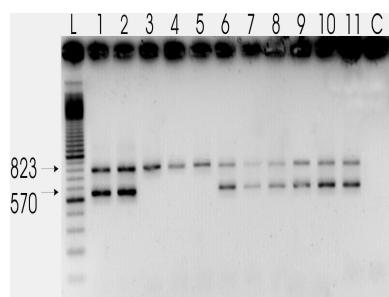


Figure 1

Electrophoresis profile of the genotypes identified at *Vf* locus in Liberty, Florina cultivars and their seedlings, with AL-07 primer

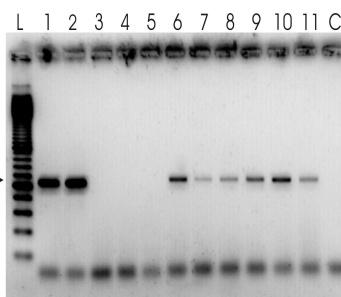


Figure 2

Electrophoresis profile of the genotypes identified at *Vf* locus in Liberty, Florina cultivars and their seedlings, with AM-19 primer

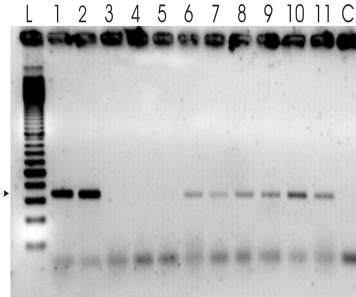


Figure 3

Electrophoresis profile of the genotypes identified at *Vf* locus in Liberty, Florina cultivars and their seedlings, with U1<sub>400</sub> primer

Figure 2 describes the 526 bp fragment of AM-19 (*Vf* allele) is in coupling with the resistant allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Liberty heterozygous scab resistant parent with genotype (*Vfvf*); and in column 2 is represented Florina heterozygous scab resistant parent with genotype (*Vfvf*). Columns 3, 4, 5 represented seedlings of this combination (9811 Liberty x Florina), with recessive homozygous genotypes (*vfvf*). Columns 6, 7, 8, 9, 10, 11, are represented by the seedlings of the same combination with heterozygous genotype (*Vfvf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 3 describes the 338 bp fragment of U1<sub>400</sub>. In column 1 is represented the electrophoresis profile of Liberty heterozygous scab resistant parent with genotype (*Vfvf*); and in column 2 is represented Florina heterozygous scab resistant parent with genotype (*Vfvf*). Columns 3, 4, 5 are represented by the seedlings of this combination (9811 Liberty x Florina), with recessive homozygous genotypes (*vfvf*). Columns 6, 7, 8, 9, 10, 11, are represented by the seedlings of the same combination with heterozygous genotype (*Vfvf*). L is a 100 bp ladder molecular weight marker and C is negative control.

The result for the combination 9812 Starkrimson x Golden Spur is present in Figure 4 and was obtained just with AL-07 primer.

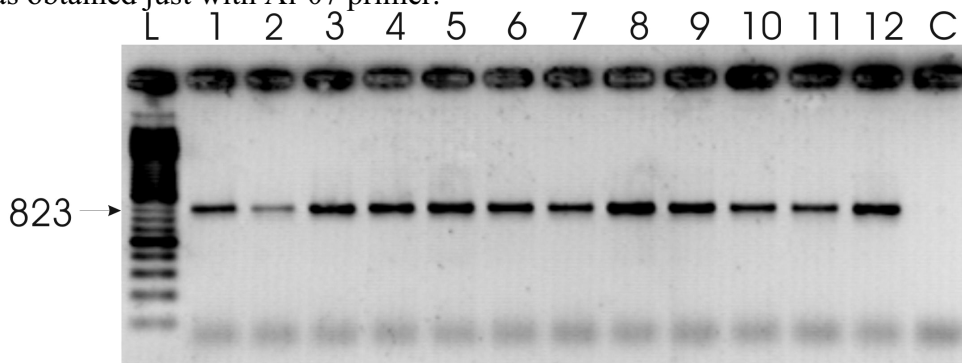


Figure 4. Electrophoresis profile of the genotypes identified at *Vf* locus in Starkrimson and Golden Spur cultivars and their seedlings with AL-07 primer

In Figure 4, the AL-07 823 bp (*vf*) alleles are in coupling with susceptible allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Starkrimson scab susceptible parent with recessive homozygous genotype (*vf/vf*) and in columns 2 is represented Golden Spur scab susceptible parent with recessive homozygous genotype (*vf/vf*). The seedlings of this combination 9812 Starkrimson x Golden Spur from columns 3, 4, 5, 6, 7 are phenotypic scab susceptible and in columns 8, 9, 10,11,12, the seedlings are phenotypic scab resistant, all of them had recessive homozygous genotypes (*vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Because all the seedlings analysed with the AL-07 were recessive homozygous (*vf/vf*), in the electrophoresis profiles obtained with the other two primers AM-19 and U1<sub>400</sub> the bands were absent.

For the combination 9813 Starkrimson x Florina results of the molecular analysis with the primers AL-07, AM-19 and U1<sub>400</sub> are presented in Figure 5, 6 and 7.

In Figure 5, the AL-07 570 bp (*Vf*) and 823 bp (*vf*) alleles are in coupling with, respectively, the resistant and susceptible allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Starkrimson recessive homozygous scab susceptible parent with genotype (*vf/vf*); and in columns 2 is represented Florina heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6 represented seedlings of this combination (9813 Starkrimson x Florina), with recessive homozygous genotypes (*vf/vf*). Column 7 is represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

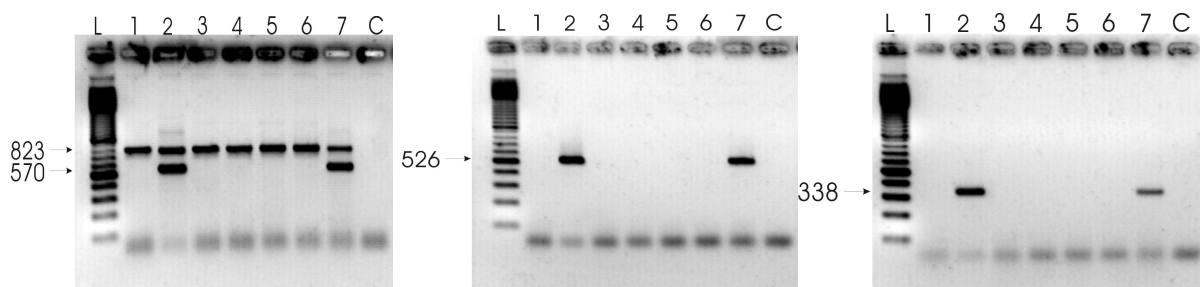


Figure 5

Electrophoresis profile of the genotypes identified at *Vf* locus in Starkrimson, Florina cultivars and their seedlings, with AL-07 primer

Figure 6

Electrophoresis profile of the genotypes identified at *Vf* locus in Starkrimson, Florina cultivars and their seedlings, with AM-19 primer

Figure 7

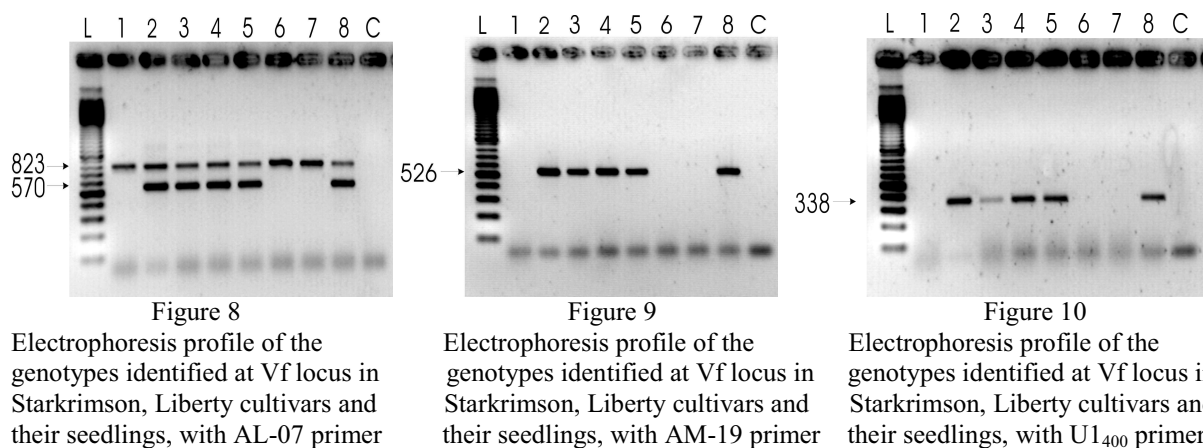
Electrophoresis profile of the genotypes identified at *Vf* locus in Starkrimson, Florina cultivars and their seedlings, with U1<sub>400</sub> primer

Figure no. 2 describes the 526 bp fragment of AM-19 (*Vf* allele) is in coupling with the resistant allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Starkrimson recessive homozygous scab susceptible parent with genotype (*vf/vf*) and in columns 2 is represented Florina heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6 represented seedlings of this combination (9813 Starkrimson x Florina), with recessive homozygous genotypes (*vf/vf*). Column 7 is represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 3 describes the 338 bp fragment of U1<sub>400</sub>. In column 1 is represented the electrophoresis profile of Starkrimson recessive homozygous scab susceptible parent with genotype (*vf/vf*) and in columns 2 is represented Florina heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6 are represented by the seedlings of this combination (9813 Starkrimson x Florina), with recessive homozygous genotypes (*vf/vf*). Column 7 is

represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

For the combination 9814 Starkrimson x Liberty results of the molecular analysis with the primers AL-07, AM-19 and U1<sub>400</sub> are presented in Figure 8, 9 and 10.



In Figure 8, the AL-07 570 bp (*Vf*) and 823 bp (*vf*) alleles are in coupling with, respectively, the resistant and susceptible allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Starkrimson recessive homozygous scab susceptible parent with genotype (*vf/vf*); and in columns 2 is represented Liberty heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 8 represented seedlings of this combination (9814 Starkrimson x Liberty), with heterozygous genotype (*Vf/vf*). Columns 6 and 7 are represented by the seedlings of the same combination with recessive homozygous genotypes (*vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 9 describes the 526 bp fragment of AM-19 (*Vf* allele) is in coupling with the resistant allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Starkrimson recessive homozygous scab susceptible parent with genotype (*vf/vf*) and in columns 2 is represented Liberty heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 8 represented seedlings of this combination (9814 Starkrimson x Liberty), with heterozygous genotype (*Vf/vf*). Columns 6 and 7 are represented by the seedlings of the same combination with recessive homozygous genotypes (*vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 10 describes the 338 bp fragment of U1<sub>400</sub>. In column 1 is represented the electrophoresis profile of Starkrimson recessive homozygous scab susceptible parent with genotype (*vf/vf*) and in columns 2 is represented Liberty heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 8 represented seedlings of this combination (9814 Starkrimson x Liberty), with heterozygous genotype (*Vf/vf*). Columns 6 and 7 are represented by the seedlings of the same combination with recessive homozygous genotypes (*vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

For the combination 9817 Golden Spur x Florina results of the molecular analysis with the primers AL-07, AM-19 and U1<sub>400</sub> are presented in Figure 11, 12 and 13.

In Figure 11, the AL-07 570 bp (*Vf*) and 823 bp (*vf*) alleles are in coupling with, respectively, the resistant and susceptible allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Golden Spur recessive homozygous scab susceptible parent with genotype (*vf/vf*); and in columns 2 is represented Florina heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6, 7 represented seedlings of this combination (9817 Golden Spur x Florina), with recessive homozygous genotypes (*vf/vf*).

Columns 8, 9, 10, 11, 12 are represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

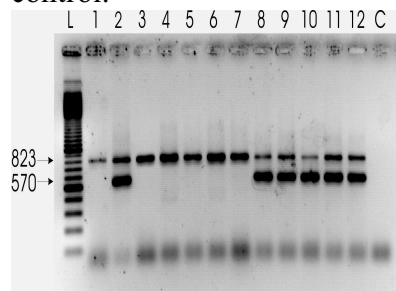


Figure 11  
Electrophoresis profile of the genotypes identified at *Vf* locus in Golden Spur, Florina cultivars and their seedlings, with AL-07 primer

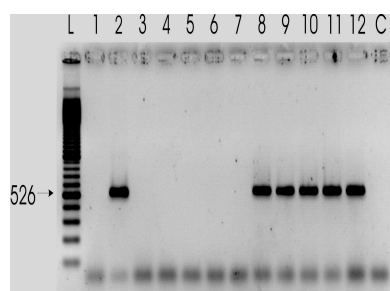


Figure 12  
Electrophoresis profile of the genotypes identified at *Vf* locus in Golden Spur, Florina cultivars and their seedlings, with AM-19 primer

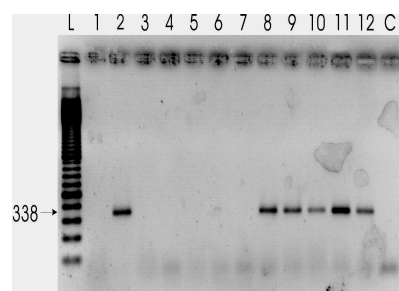


Figure 13  
Electrophoresis profile of the genotypes identified at *Vf* locus in Golden Spur, Florina cultivars and their seedlings, with U1<sub>400</sub> primer

Figure no. 12 describes the 526 bp fragment of AM-19 (*Vf* allele) is in coupling with the resistant allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Golden Spur recessive homozygous scab susceptible parent with genotype (*vv/vv*) and in column 2 is represented Florina heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6, 7 represented seedlings of this combination (9817 Golden Spur x Florina), with recessive homozygous genotypes (*vv/vv*). Columns 8, 9, 10, 11, 12 are represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 13 describes the 338 bp fragment of U1<sub>400</sub>. In column 1 is represented the electrophoresis profile of Golden Spur recessive homozygous scab susceptible parent with genotype (*vv/vv*) and in column 2 is represented Florina heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6, 7 represented seedlings of this combination (9817 Golden Spur x Florina), with recessive homozygous genotypes (*vv/vv*). Columns 8, 9, 10, 11, 12 are represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

For the combination 9818 Golden Spur x Liberty results of the molecular analysis with the primers AL-07, AM-19 and U1<sub>400</sub> are presented in Figure 14, 15 and 16.

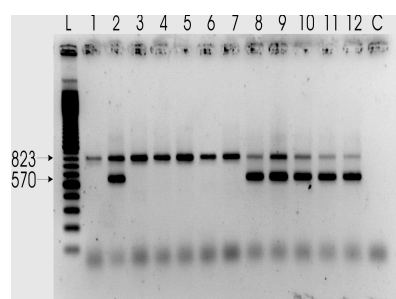


Figure 14  
Electrophoresis profile of the genotypes identified at *Vf* locus in Golden Spur, Liberty cultivars and their seedlings, with AL-07 primer

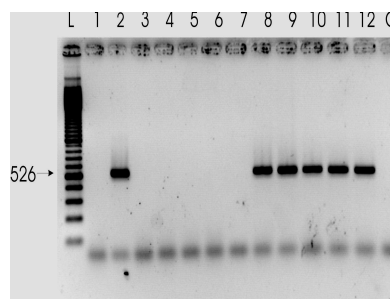


Figure 15  
Electrophoresis profile of the genotypes identified at *Vf* locus in Golden Spur, Liberty cultivars and their seedlings, with AM-19 primer

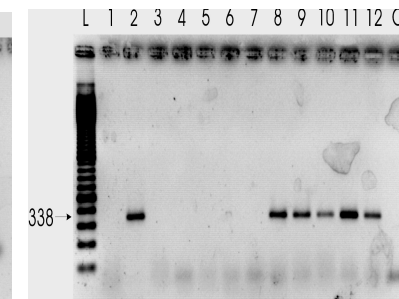


Figure 16  
Electrophoresis profile of the genotypes identified at *Vf* locus in Golden Spur, Liberty cultivars and their seedlings, with U1<sub>400</sub> primer

In figure 14, the AL-07 570 bp (*Vf*) and 823 bp (*vf*) alleles are in coupling with, respectively, the resistant and susceptible allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Golden Spur recessive homozygous scab susceptible parent with genotype (*vf/vf*); and in columns 2 is represented Liberty heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6, 7 represented seedlings of this combination (9818 Golden Spur x Liberty), with recessive homozygous genotypes (*vf/vf*). Columns 8, 9, 10, 11, 12 are represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 15 describes the 526 bp fragment of AM-19 (*Vf* allele) is in coupling with the resistant allele of the *Vf* gene for scab resistance. In column 1 is represented the electrophoresis profile of Golden Spur recessive homozygous scab susceptible parents with genotype (*vf/vf*) and in columns 2 is represented Liberty heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6, 7 represented seedlings of this combination (9818 Golden Spur x Liberty), with recessive homozygous genotypes (*vf/vf*). Columns 8, 9, 10, 11, 12 are represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Figure no. 16 describes the 338 bp fragment of U1<sub>400</sub>. In column 1 is represented the electrophoresis profile of Golden Spur recessive homozygous scab susceptible parent with genotype (*vf/vf*) and in columns 2 is represented Liberty heterozygous scab resistant parent with genotype (*Vf/vf*). Columns 3, 4, 5, 6, 7 represented seedlings of this combination (9818 Golden Spur x Liberty), with recessive homozygous genotypes (*vf/vf*). Columns 8, 9, 10, 11, 12 are represented by the seedlings of the same combination with heterozygous genotype (*Vf/vf*). L is a 100 bp ladder molecular weight marker and C is negative control.

Table 7

The different genotypes of the four apple cultivars used as genitors and sixty seedlings for *Vf* gene, obtained with the primers AL-07, AM-19, U1<sub>400</sub>

No. Sample	Cultivar/ Hybrid Combinations	Phenotype	Primers*			Genotype
			AL-07	AM-19	U1 <sub>400</sub>	
1	Starkrimson	Susceptible	+	-	-	<i>vf/vf</i>
2	Golden Spur	Susceptible	+	-	-	<i>vf/vf</i>
3	Liberty	Resistant	++	+	+	<i>Vf/vf</i>
4	Florina	Resistant	++	+	+	<i>Vf/vf</i>
5	Liberty x Florina	Susceptible	+	-	-	<i>vf/vf</i>
6	Liberty x Florina	Susceptible	+	-	-	<i>vf/vf</i>
7	Libertyx Florina	Susceptible	+	-	-	<i>vf/vf</i>
8	Liberty x Florina	Susceptible	++	+	+	<i>Vf/vf</i>
9	Liberty x Florina	Susceptible	++	+	+	<i>Vf/vf</i>
10	Liberty x Florina	Resistant	++	+	+	<i>Vf/vf</i>
11	Liberty x Florina	Resistant	++	+	+	<i>Vf/vf</i>
12	Liberty x Florina	Resistant	++	+	+	<i>Vf/vf</i>
13	Liberty x Florina	Resistant	++	+	+	<i>Vf/vf</i>
14	Liberty x Florina	Resistant	++	+	+	<i>Vf/vf</i>
15	Starkrimson x Golden Spur	Susceptible	+	-	-	<i>vf/vf</i>
16	Starkrimson x Golden Spur	Susceptible	+	-	-	<i>vf/vf</i>
17	Starkrimson x Golden Spur	Susceptible	+	-	-	<i>vf/vf</i>
18	Starkrimson x Golden Spur	Susceptible	+	-	-	<i>vf/vf</i>
19	Starkrimson x Golden Spur	Susceptible	+	-	-	<i>vf/vf</i>
20	Starkrimson x Golden Spur	Resistant	+	-	-	<i>vf/vf</i>

21	Starkrimson x Golden Spur	Resistant	+	-	-	<i>vf/f</i>
22	Starkrimson x Golden Spur	Resistant	+	-	-	<i>vf/f</i>
23	Starkrimson x Golden Spur	Resistant	+	-	-	<i>vf/f</i>
24	Starkrimson x Golden Spur	Resistant	+	-	-	<i>vf/f</i>
25	Starkrimson x Florina	Susceptible	+	-	-	<i>vf/f</i>
26	Starkrimson x Florina	Susceptible	+	-	-	<i>vf/f</i>
27	Starkrimson x Florina	Susceptible	+	-	-	<i>vf/f</i>
28	Starkrimson x Florina	Susceptible	+	-	-	<i>vf/f</i>
29	Starkrimson x Florina	Susceptible	+	-	-	<i>vf/f</i>
30	Starkrimson x Florina	Resistant	++	+	+	<i>Vf/f</i>
31	Starkrimson x Florina	Resistant	++	+	+	<i>Vf/f</i>
32	Starkrimson x Florina	Resistant	++	+	+	<i>Vf/f</i>
33	Starkrimson x Florina	Resistant	++	+	+	<i>Vf/f</i>
34	Starkrimson x Florina	Resistant	++	+	+	<i>Vf/f</i>
35	Starkrimson x Liberty	Susceptible	+	-	-	<i>vf/f</i>
36	Starkrimson x Liberty	Susceptible	++	+	+	<i>Vf/f</i>
37	Starkrimson x Liberty	Susceptible	++	+	+	<i>Vf/f</i>
38	Starkrimson x Liberty	Susceptible	+	-	-	<i>vf/f</i>
39	Starkrimson x Liberty	Susceptible	++	+	+	<i>Vf/f</i>
40	Starkrimson x Liberty	Resistant	++	+	+	<i>Vf/f</i>
41	Starkrimson x Liberty	Resistant	+	-	-	<i>vf/f</i>
42	Starkrimson x Liberty	Resistant	++	+	+	<i>Vf/f</i>
43	Starkrimson x Liberty	Resistant	+	-	-	<i>vf/f</i>
44	Starkrimson x Liberty	Resistant	++	+	+	<i>Vf/f</i>
45	Golden Spur x Florina	Susceptible	+	-	-	<i>vf/f</i>
46	Golden Spur x Florina	Susceptible	+	-	-	<i>vf/f</i>
47	Golden Spur x Florina	Susceptible	+	-	-	<i>vf/f</i>
48	Golden Spur x Florina	Susceptible	+	-	-	<i>vf/f</i>
49	Golden Spur x Florina	Susceptible	+	-	-	<i>vf/f</i>
50	Golden Spur x Florina	Resistant	++	+	+	<i>Vf/f</i>
51	Golden Spur x Florina	Resistant	++	+	+	<i>Vf/f</i>
52	Golden Spur x Florina	Resistant	++	+	+	<i>Vf/f</i>
53	Golden Spur x Florina	Resistant	++	+	+	<i>Vf/f</i>
54	Golden Spur x Florina	Resistant	++	+	+	<i>Vf/f</i>
55	Golden Spur x Liberty	Susceptible	+	-	-	<i>vf/f</i>
56	Golden Spur x Liberty	Susceptible	+	-	-	<i>vf/f</i>
57	Golden Spur x Liberty	Susceptible	+	-	-	<i>vf/f</i>
58	Golden Spur x Liberty	Susceptible	+	-	-	<i>vf/f</i>
59	Golden Spur x Liberty	Susceptible	+	-	-	<i>vf/f</i>
60	Golden Spur x Liberty	Resistant	++	+	+	<i>Vf/f</i>
61	Golden Spur x Liberty	Resistant	++	+	+	<i>Vf/f</i>
62	Golden Spur x Liberty	Resistant	++	+	+	<i>Vf/f</i>
63	Golden Spur x Liberty	Resistant	++	+	+	<i>Vf/f</i>
64	Golden Spur x Liberty	Resistant	++	+	+	<i>Vf/f</i>

Legend\*

+ - - genotype *vf/f*

++ ++ genotype *Vf/f*

## CONCLUSIONS

The molecular analysis of all this apple cultivars and hybrids was done with one co-dominant primer (AL-07) and two dominant primers (AM-19 and U1<sub>400</sub>) and the results obtained with all of them confirmed the presence or absence of *Vf* gene. The co-dominant marker AL-07 was very useful to discriminated homozygous from heterozygous plants for the *Vf* gene.

In this study, the results obtained at the genetic level using specific primers for *Vf* gene confirmed the resistance and the susceptibility of the plants, phenotypically selected by presence or absence of infection symptoms. Because the Starkrimson and Golden Spur are recessive homozygous scab susceptible parents, the seedlings of this combination didn't have any segregation ratio for *Vf* gene, but they presented an ample variability for their response to scab attack, which could explain by polygenic effect of the genes. In the others five combinations the segregation ratio of seedlings for their response to apple scab attack, and for *Vf* gene, was situated in Mendelian ratio 50% scab resistant : 50% scab susceptible. In combinations resistant x resistant (Liberty x Florina) there were identified susceptible and resistant offspring, but at last, only with heterozygous for *Vf* gene (*Vf**vf*) and no one with dominant homozygous genotypes (*VfVf*).

The classical selection methods for apple scab resistance can be very much improved using genetic markers tightly linked to the *Vf* resistance gene. Molecular markers linked to important phenotypic traits may become an important tool in shortening the length of selection process by reducing time and costs of entire breeding programme. Marker assisted selection of juvenile plants can facilitate the breeding programme for apple scab resistance.

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## REZUMAT

### UTILIZAREA SELECȚIEI ASISTATĂ DE MARKERI MOLECULARI PENTRU DETERMINAREA REZISTENȚEI MĂRULUI LA ATACUL DE RAPĂN

Rapănul mărului, produs de ciuperca *Venturia inaequalis*, este o boala larg răspândită, având un impact negativ asupra producției și calității acesteia. Ca atare, programele de ameliorare a mărului vizează crearea unor soiuri noi, rezistente la atacul de rapăn. În acest sens, se încearcă transferarea unor gene de rezistență în diferite soiuri comerciale de măr, cea mai folosită fiind gena *Vf*, provenită de la *Malus floribunda* 821. În studiul de față s-a utilizat selecția asistată de markeri moleculari, pentru analiza constituției genetice la patru soiuri de măr utilizate ca genitori și a hibridilor proveniți din ele. Analizele moleculare realizate cu markerii AL-07, AM-19 și U1<sub>400</sub> au pus în evidență prezența genei *Vf*, la hibridii proveniți din soiurile cu rezistență genetică (Liberty și Florina), indiferent dacă hibridii F<sub>1</sub> au provenit din încrucișări între cele două soiuri rezistente, sau din încrucișări dintre acestea și soiuri fără sursa de rezistență *Vf*, respectiv Starkrimson și Golden spur. Dintre soiurile genitoare, cele rezistente, Liberty și Florina, au fost identificate ca heterozigote pentru gena *Vf* (*Vfvf*), iar cele sensibile, ca homozigot recesive (*vfvf*). Prin selecție fenotipică, din fiecare combinație hibridă au fost aleși zece descendenți F<sub>1</sub>, dintre care cinci rezistenți și cinci sensibili la atacul de rapăn pe frunze. Au rezultat 60 de hibridi care au fost analizați la nivel de ADN, dintre aceștia 28 fiind rezistenți și având genotipul heterozigot (*Vfvf*), iar 32 sensibili și cu genotipul homozigot recesiv (*vfvf*). În combinația cu soiuri sensibile (Starkrimson x Golden Spur), toți hibridii au fost homozigoți recesivi (*vfvf*), eventuala rezistență fenotipică a unora fiind explicată prin efectele de aditivitate ale poligenelor. În descendența soiurilor rezistente (Liberty x Florina), s-au obținut hibridi sensibili (*vfvf*) și rezistenți, ultimii fiind identificați doar ca heterozigoți pentru gena *Vf* (*Vfvf*).