Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 1, 2022
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EVIDENCE OF THE PRESENCE OF APPLE SCAB RESISTANCE *VF* GENE OF SOME LOCAL APPLE CULTIVARS FROM TRANSYLVANIA

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Abstract

Apple is one of the most important crops in temperate areas, and it is situated on the second place, after plum, in Romania. The scab caused by Venturia inaequalis is a widespread disease in apple crop, which produces considerable economic losses. The aim of the study was to identify the presence of the Vf gene in four local apple cultivars ('Goldprim', 'Dany', 'Doina' and 'Alex'), obtained by the FRDS Bistrita. The investigated cultivars are known to be resistant to scab, but their selection was made by classical methods which do not guarantee the presence of Vf gene. To get an accurate confirmation of the Vf resistance mechanism in the genome of the cultivars, a molecular tool was used. Thus, the mentioned apple cultivars were tested by using the Marker Assisted Selection (MAS) method, with three pairs of specific markers: AL07, which allows the producing two bands corresponding to Vf-dominant, and vf-recessive respectively; the other two markers are AM19 and $U1_{400}$ as dominants (Vf). According to the results obtained, the Vf resistance genes were identified as heterozygous (Vfvf) in all four local apple cultivars tested.

Key words*: apple, disease, molecular marker, scab, Vf gene.*

INTRODUCTION

Apple cultivation is one of the most widespread crops in the temperate areas of the world. The variability of the genus *Malus* allows adaptation of the species to different geographical areas. Being a perennial species, apple is long-term crop and requires a number of specific cultivation technologies (Kellerhals et al., 2009; Muneer et al., 2017). In apple crops, the list of diseases caused by fungi, bacteria, viruses and phytoplasmas, include about 80 such diseases and physiological imbalances, to which must be added 64 species of insects and 8 species of nematodes (Köller & Parker, 1989). Certain pests and diseases are constantly dynamic, with an increasing trend, but not all are present in the same growing areas (Köller and Parker, 1989). One of the most widespread and damaging diseases in apple species is scab caused by the ascomycete fungus *Venturia inaequalis* (Luo et al., 2020). For this reason, breeding programs are aiming to obtain disease resistant/tolerant cultivars (Cordea, 2014). Thus, through them, the number of crop protection treatments can be significantly reduced. There are two types of apple resistance to scab: monogenic and polygenic (Durel et al., 2003). An important sources of monogenic apple resistance to scab is given by the *Vf* gene (named after the new nomenclature *Rvi*6), derived from *Malus floribunda* clone 821 (Bus et al., 2009). There are plenty of apple cultivars obtained in the past from controlled crosses based on empirical selection, and considered resistant to disease, because one of the genitors was known to have the *Vf* gene in the genome.

In the field of plant breeding, new molecular methods allow on the one hand the identification of genes of interest at the seedling stage of plants, and on the other hand reduced costs and shorten selection process (Soriano, 2020). In this context, selection efficiency is a key issue in the proper choice of cultivars to be used as parents in breeding

programs (Kellerhals et al., 2009). Tartarini et al. (2000) concluded that classical selection does not prove sufficient accuracy in plant resistance of trees to scab compared to the Marker Assisted Selection. The aim of the present study was to verify the presence of the *Vf* gene in four local apple cultivars breeded at FRDS Bistrita supposed to carry the *Vf* gene.

MATERIALS AND METHODS

The material used for the experiment consists of four local apple cultivars ('Goldprim', 'Dany', 'Doina' and 'Alex') registered by Fruit Research & Development Station Bistrita (Table 1).
In this study, the Florina cultivar (*Vfvf* - Pătrașcu, et al., 2006) was used as a positive control and the Golden delicious (*vfvf* - Sestraș, 2004) variety as a negative control.
Following a classic selection process, which was initially carried out empirically, the cultivars mentioned are known to be resistant to scab (Braniște et al., 2007), but this is not a guarantee of the presence of the *Vf* gene.

Table 1. List of cultivars used in experience

Cultivar	Genitors	Year of approval
'Goldprim'	♀Golden delicious x ♂Prima	2003
'Dany'	♀Jonathan irradiated with P33 x ♂Prima	2003
'Doina'	♀Jonathan x ♂Prima	2003
'Alex'	♀Golden delicious x ♂BN 33/39	2004

Therefore, to accurately determine the presence or absence of the *Vf* gene conferring scab resistance, PCR (Polymerase Chain Reaction) analyses were performed by using specific markers: two pairs of dominant primers (AM19, $U1_{400}$) and another codominant primer (AL07) to distinguish between homozygous and heterozygous genotypes (Table 2).

Table 2. List of primers used

No.	Marker name	Primer sequences 5' to 3'	No. nucleotide	Primer type	Fragment size (bp)	References
1	AL07	F-TGGAAGAGAGATCCAGAAAGTG	22	Codominant	570 (*Vf*)	Khajuria et al., 2014
		R-CATCCCTCCACAAATGCC	18		823 (*vf*)	Tartarini et al., 1999
2	AM19	F-CGTAGAACGGAATTTGACAGTG	22	Dominant	526 (*Vf*)	Khajuria et al., 2014
		R-GACAAAGGGCTTAAGTGCTCC	21			Tartarini et al., 1999
3	$U1_{400}$	F-GTAAAGCAAGCACTTCAACG	20	Dominant	338 (*Vf*)	Hemmat et al., 1998
		R-GTAAAATAGATGTGTGGGTAGC	22			

DNA extraction protocol

DNA extraction was performed from lyophilized leaves in calcium chloride stored at 4°C. Bioline's Plant DNA Extraction Kit was used to isolate DNA according to the protocol indicated by the manufacturer. Finally, 50 µl of pure DNA was obtained for each sample. Measurements of DNA concentration and purity were performed using the NANODROP 2000c spectrophotometer.

DNA amplification

The preparation of DNA amplification mix was performed using MyTaq Red Mix from Bioline, obtained a final volume of 25 µl/tube for each sample. DNA amplification was performed in an Eppendorf Mastercycler. For primers AL07 and AM19, the cycling parameters used were as follows: 1 min at 95°C - initial denaturation; 35 cycles, each consisting of: 1 min at 94°C - denaturation, 1 min at 60°C - annealing, 2 min at 72°C - extension, and 10 min at 72°C - final extension. For primer $U1_{400}$ the cycling parameters used were as follows: 1 min at 95°C - initial denaturation, 2 min at 94°C - denaturation, 2 min at 69°C - annealing, 2 min at 72°C - extension. 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. The final extension cycle was applied for 10 minutes at 72°C. The final concentration of primers in the reaction was 1 µM.

Migration of amplified products

The amplified fragments were fractionated in 1,5% agarose gel in a 1X TAE buffer for 50 min, stained by RedSafe Nucleic Acid Staining Solution. 100 bp DNA Ladder RTU was used

as a size marker. Bands were visualised using Quantity One 1-D Analysis Software system under UV light.

RESULTS AND DISCUSSIONS

As expected, due to the fact that the male genitors of each apple cultivar studied (Table 1) carry the *Vf* gene (Braniște et al., 2007), it was also transmitted to the progenies.
Thus, according to the molecular tests performed, all four tested cultivars ('Goldprim', 'Dany', 'Doina', and 'Alex') had the scab resistance gene. The specific fragments of *Vf* gene (*Rvi6*) were amplified by all three molecular markers. Thus, both the 570 bp fragment of dominant gene and the 823 bp of recessive gene were amplified by the codominant primer AL07.
Using the other two sets of dominant primers, PCR reaction amplified fragments of 526 bp with the AM19 marker, and of 338 bp with the $U1_{400}$ marker, respectively, identical with molecular size of positive controls (Figure 1).

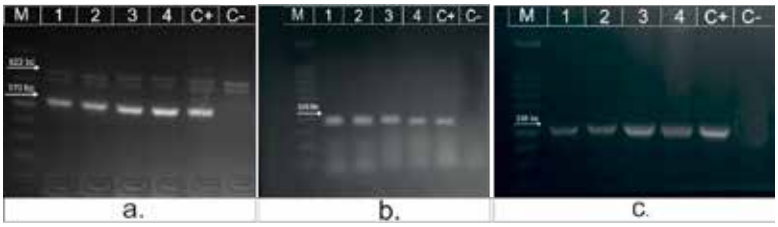

Figure 1. Electrophoresis profile of the genotypes identified at *Vf* locus in cultivar with AL07 primer set (a), AM19 primer set (b) and $U1_{400}$ primer set (c)
M – Marker; 1 - 'Goldprim'; 2 - 'Dany'; 3 - 'Doina'; 4 - 'Alex'; 'Florina' (C+); 'Golden delicious' (C-)

The bands obtained on gels were represented in the form of binary data, being noted by "+" for the presence and "-" for the absence of the *Vf* gene for each cultivar depending on the marker used (Table 3). According to the results obtained, all the studied cultivars are heterozygous for the resistance gene *Vf*.

Table 3. Cultivars investigated for scab resistance

Cultivar	AL07		AM19	$U1_{400}$	Detected genotype
	Vf	*vf*	*Vf*	*Vf*	
'Goldprim'	+	+	+	+	*Vfvf*
'Dany'	+	+	+	+	*Vfvf*
'Doina'	+	+	+	+	*Vfvf*
'Alex'	+	+	+	+	*Vfvf*
'Florina' (C+)	+	+	+	+	*Vfvf*
'Golden delicious' (C-)	-	+	-	-	*vfvf*

Similar results in confirming the presence of the *Vf* gene in other local varieties of old apples or apple hybrids have been reported in other studies. Militaru et al. (2020) studied the presence of the *Vf* gene in some local varieties that are known to be resistant to crust. Bivolariu et al. (2021) used molecular selection techniques on a sample of seedling apple hybrids. In the mentioned studies, the same sets of specific markers (AL07, AM19 and U1400) were used to identify the *Vf* apple resistance gene in different apple varieties or hybrids.

CONCLUSIONS

The studies confirmed the presence of the *Vf* gene, which confers scab resistance (monogenic resistance) to all analyzed varieties, using the MAS technique. The *Rvi6* gene was amplified by the three markers used. Therefore, these cultivars can be used as donors of *Vf* resistance gene in further apple breeding programs.

ACKNOWLEDGEMENTS

This research work was carried out with the support by Romanian Ministry of Agriculture, contract ADER 7.2.2 / 2019.

REFERENCES

Bivolariu (Guzu), G., Zagrai, I., Zagrai, L., Cordea, M., & Moldovan, C. (2021). Molecular screening of some apple progenies for detection *Vf* gene using Marker Assisted Selection. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, 78(2), 36–40.

Braniște, N., Budan, S., Butac, M., Militaru, M., (2007). *Varieties of trees, fruit bushes and strawberries created in Romania.* Pitești, RO: Paralela 45 House.

Bus, V., Rikkerink, E., Aldwinckle, H. S., Caffier, V., Durel, C.-E., Gardiner, S., Gessler, C., Groenwold, R., Laurens, F., Le, C. B., Luby, J., MacHardy, W., Meulenbroek, B., Kellerhals, M., Parisi, L., Patocchi, A., Plumme,r K., Schouten, H. J., Tartarini, S., & Van de Weg, E. A. (2009). Proposal for the nomenclature of *Venturia inaequalis* races. *Acta Hort. (ISHS)*, 814: 739–746.

Cordea, M. I. (2014). Horticultural plant breeding – practical works. RO: AcademicPres Cluj-Napoca.

Durel, C. E., Parisi, L., Laurens, F., Van de Weg, We., Liebhard, R., & Jourjon, M. F. (2003). Genetic dissection of partial resistance to race 6 of *Venturia inaequalis* in apple. *Genome* 46:224–234.

Hemmat, M., Weeden, N.F., Aldwinckle., H. S., & Brown S. K. (1998). Molecular markers for the scab resistance (Vf) region in apple. *J. Amer. Soc. Hort*, SCI., 123 (6), 992–996.

Kellerhals, M., Spuhler, M., Duffy, B., Patocchi, A., & Frey, J. E. (2009). Selection Efficiency in Apple Breeding. *Acta Horticulturae*, (814), 177–184.

Khajuria, Y. P., Kaul, S., Wafai, B. A., & Dhar, M. K. (2014). Screening of apple germplasm of Kashmir Himalayas for scab resistance genes. *Indian Journal of Biotechnology* 13; 448–454.

Köller, W., & Parker, D. M. (1989). Purification and characterization of cutinase from *Venturia inaequalis*. *Phytopathology*, 79, 278–283.

Luo, F., Evans, K., Norelli, J. L., Zhang, Z., & Peace, C. (2020). Prospects for achieving durable disease resistance with elite fruit quality in apple breeding. *Tree Genetics & Genomes*, 16(1).

Militaru, M., Sturzeanu, M., & Iancu, A. (2020). Molecular screening of some Romanian apple cultivars for scab resistance genes. *Fruit Growing Research, XXXVI*, 5–11.

Muneer, A. S., Bhat K. M., Mir, J. I., Sajad, Un Nabi, Bhat, M. A., Hilal, A., Wajida, S., Shafia Z., Sumaira J., & Waseem, H. R. (2017). Phenotypic and molecular screening for diseases resistance of apple cultivars and selections against apple scab (*Venturia inaequalis*). *International Journal of Chemical Studies* 5(4): 1107–1113.

Pătrașcu, B, Pamfil, D,, Sestraș, R,, Botez, C,, Gaboreanu, I,, Barbos, A,, Qin, C,, Rusu, R,, Bondrea, I,, Dirle, E., (2006). Marker assisted selections for response attack of *Venturia inaequalis* in different apple genotypes. *Notulae Botanicae Horti Agrobotanici 34*. 2006; 121–133.

Sestraș, R., (2004). *Improvement of horticultural species*. Cluj Napoca, RO: AcademicPres House

Soriano, J. M. (2020). Molecular Marker Technology for Crop Improvement. *Agronomy*, 10(10), 1462.

Tartarini, S., Sansavini, S., Vinatzer, B., & Gennari, F. C. D. (2000). Efficiency of marker assisted selection (MAS) for the *Vf* scab resistance gene. *Acta Hortic* 538: 549–5.

Tartarini, S., Gianfranceschi, L., Sansavini, S., & Gessler, C. (1999). Development of reliable PCR markers for the selection of the *Vf* gene confering scab resistance in apple. *Plant Breeding*, 118, 183–186.