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SCREENINGUL MOLECULAR AL UNOR SOIURI STRĂINE ȘI ROMÂNEȘTI UTILIZATE ÎN PROGRAMELE DE AMELIORARE DIN ROMÂNIA

THE MOLECULAR SCREENING OF SOME FOREIGN AND ROMANIAN VARIETIES USED IN BREEDING PROGRAMS IN ROMANIA

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Cuvinte cheie: măr, diversitate genetică, *Rvi6, Rvi2, Rvi8, Rvi5*, tehnica SSR, gene de rezistenţă.
Key words: apple, genetic diversity, *Rvi6, Rvi2, Rvi8, Rvi5*, SCAR technique, resistance genes.

Abstract

In Romania, 70 improved varieties were created starting in 1996 and until now. Some of them show resistance to scab, being carriers of some genes described in various specialized works, but, for some time, a decrease in resistance to the pathogen *Venturia inaequalis* has been observed in varieties known to be resistant, so must be initiated a reorganization of the hybridization in breeding programs. In this study, a molecular screening was performed for some of the parents that were used to create the varieties in breeding programs from Romania. The apple varieties introduced in this study were differentiated with the following molecular markers: AL07, AM19, *Vfc* for the *Rvi6* gene; OPL19 for *Rvi2* and *Rvi8* gene; AD13 for the *Rvi4* gene, respectively OPB12 and Hi07ho2 for the *Rvi5* gene, some of the varieties being identified as carrier more than two resistance genes ('Florina', 'Romus 3', 'Romus 4', 'Romus 5', 'Pionier' and 'Starkrimson').

1. Introduction

The first research, carried out in Romania, by applying molecular analysis techniques on apple tree resistance to scab was started in 2006-2008, at USAMV Cluj-Napoca, by a team of researchers, when realized a molecular screening of 64 hybrids resulting from crosses between varieties was carried out: 'Liberty' x 'Florina', 'Starkrimson' x 'Golden Spur', 'Starkrimson' x 'Florina', 'Starkrimson' x 'Liberty', 'Golden Spur' x 'Florina' and 'Golden Spur' x 'Liberty'. Three molecular markers, SCAR, AL07 (co-dominant), AM19 (dominant) and U1400 (dominant) were used in this study to evidence the *Vf* gene.

Research results showed a Mendelian-like segregation ratio, in which 50% of the hybrids were carriers of the *Vf* gene, and 50% were susceptible, not being carriers of the *Vf* gene (Bodea et al., 2007-2008; Pătrașcu et al., 2006).

At the Research Institute for Fruit Growing Pitesti-Romania, in the period 2020-2022, valuable results have been obtained for 48 varieties created at the main research centers in the country and 2 local varieties, following research related to the identification of several genes for resistance to scab, when three SCAR markers for the *Rvi6* (*Vf*) gene were used: AL07, AM19, *Vfc*, a marker for the *Vr* (*Rvi2*+*Rvi8*) gene: OPL19, a marker for the *Rvi5* (*Vm*) gene: OPB12 and a marker for the *Rvi4* (*Vh4*) gene: AD13. The results showed the presence of the genes: *Rvi6* in 31 ameliorated varieties, *Rvi2* and *Rvi8* in 23 ameliorate varieties and 2 local varieties, *Rvi5* in 3 ameliorated varieties and *Rvi4* in 12 ameliorated varieties (Militaru et al., 2020).

Also in Romania, at the Research Station for Fruit Growing Bistriţa, in a study conducted on 26 hybrids obtained by crossing between varieties 'Florina' and 'Auriu de Bistrita' using markers: AL07, AM19 and U1400, it was confirmed, following the Mendelian segregation model, the presence of 14 hybrids with resistance to scab (*Vf* gene) and 12 susceptible hybrids (Bivolariu et al., 2021).

Earlier studies carried out in Italy on the evidence of the *Rvi6* gene (*Vf)* by using SCAR markers: AL07, AM19 and RAPD markers: OPAL07, OPAM19 for 616 hybrids obtained by crosses between varieties: 'Florina' and 'Nova Easygro' (491), 'Prima' and 'Golden Delicious' (40), 'Prima' and 'Jerseymac' (42), 'Prima' and 'Summerred' (27), 'Prima' and 'Florina' (8) and varieties 'Prima' and 'Priscilla' (8), it was confirmed the presence of *Vf* gene by obtaining amplified fragments corresponding to the length of PCR product as follows: 466 bp (OPAL07), 526 bp (OPAM19), 466 bp (AL07), and 526 bp (AM19), respectively (Tartarini et al., 1999).

The use of the AD13 marker, by a team of researchers at the Federal Centre for Breeding Research on Cultivated Plants, Institute of Fruit Breeding, Germany, for molecular screening of three progeny populations obtained by hybridization between varieties: 'Regia' and 'Pingo', 'Regia' and 'Pinova', respectively 'Regia' × 'Piflora', allowed the detection of the *Rvi4* gene by the existence of one amplicon of 950 bp dimension (Boudichevskaia et al., 2006).

Cheng et al. (1998) is among the first researchers to initiate studies on the presence of the *Rvi5* gene, using the OPB12 marker, in hybrid populations derived from the 'Empire' variety and the selection 'NY74828-12' the *Rvi5* gene's carrier inherited from *M.* × *atrosanguinea* 804, but also in hybrid populations obtained by hybridization between 'Royal Gala' and the selection 'OR45T132', also the *Rvi5* gene's carrier. .

Bus et al. (2005a) used markers Ch02b10, CH05e03 and CH03d01 to study the selection 'TSR34T15', considered to be the differential host of the *Vh2* (*Rvi2*) gene, obtaining amplification fragments corresponding following lengths: 122, 165, 119. Linkage of marker Ch02b10 with the *Rvi2* gene was achieved at a distance of 8 cM, while for the other two markers linkage to the gene was assessed at the distal end of the linkage group .

Gygax et al. (2004) in a molecular study of 173 hybrids derived from a cross between the selection 'A722-7' and the variety 'Golden Delicious', obtained amplified fragments using the markers: CH02c06, CH03d01 and CH05e03, which he attributed to the *Rvi11* gene: 248 (230, 236,240) bp, 115 (109, 113) bp and 150 (176, 182, 190) bp.

Molecular studies recently effectuated in Greece on 20 autochthonous varieties grown in different regions using 12 molecular markers, including 7 SCAR (OPB18, AD13, AL07, AM19, OPL19, S22, K08), 4 SSR (CH02b10, CH02c02a, CH05e03, CH-*Vf1*) and 1 PCR (Hcr*Vf2*) showed a wide genetic diversity, with the following scab resistance genes identified as *Rvi2* (18 cultivars with OPB18 and 4 cultivars with CH02b10), *Rvi4* (8 cultivars with CH02c02a and 2 cultivars with S22), *Rvi8* (9 cultivars with OPL19), *Rvi11* (5 cultivars with K08 and 1 cultivar with CH05e03) (Karapetsi et al., 2020).

Also in topical studies, Höfer et al. (2021) has contributed to improving the information on the identification of the previously obtained PCR fragments with markers: CH02b10 (122:146 bp), CH05e03 (163 bp), Hi07h02 (226:273), CH02c06 (233:245) and Ch03d01 (103:105) for TSR34T15 (*Rvi2*: CH02b10, CH05e03), 9-AR2T196 (*Rvi5*: Hi07h02) and *Malus* species selections. '*baccata var. jackii'* (*Rvi11*: Ch03d01, CH02c06).

Also of interest are the results obtained at the University of Eastern Finland, Faculty of Science and Forestry, Department of Environmental and Biological Sciences, where 38 columnar, 26 potential columnar and 16 ornamental apple selections for parks and gardens ('Dialog', 'Dzin', 'Ikaza', 'Medok', 'President', 'Valjuta', 'Vasjugan' and 'X2') have been assessed to molecular analysis for resistance to scab, using AL07 and AM19 markers. After the analysis of the result, 2 columnar, 1 potential columnar and 1 garden selection was identified with scab resistance (Bekbergen, 2016). The present work aimed to perform molecular screening of some foreign and Romanian varieties used in breeding programs in order to omit some errors related to the presence or absence of carrier genes in the descendants to be molecularly analyzed.

2. Material and methods

Twenty-six apple varieties used as parents in breeding programs in Romania were included in the study, of which two varieties are local ('Verzișoare' and 'Crețesc'), two varieties are created at the Research Station for Fruit Growing Voinești ('Frumos de Voinești', 'Pionier'), nine varieties of US origin ('Jonathan', 'Prima', 'Wagner Premiat', 'Golden Delicious', 'Rome Beauty', 'Jonagold', 'Golden Spur', 'Sir Prise', 'Starkrimson'), four varieties registered by Research Institute for Fruit Growing Pitesti ('Romus 2', 'Romus 3', 'Romus 4', 'Romus 5'), as well as' from other countries: 'McIntosh' (Canada), 'Grany Smith' (Australia), 'Belle de Boskoop' (Netherlands), 'Parmen d'or' (England), 'Mutsu' (Japan), 'Champion' (Czech Republic), 'Florina' (France) and two species (*Malus floribunda* and *Malus kaido*) (Table 1).

Three markers associated with the *Rvi6* (*Vf*) gene (AL07, AM19 and *Vfc*), one marker associated with the *Rvi4* (*Vr1*) gene and two markers associated with the *Rvi5* (*Vm*) gene (OPB12 and Hi07h02) were introduced into the study (Table 3).

DNA was extracted as described in the extraction protocol recommended by de "ISOLATE II Plant DNA Kit", Bioline and PCR-SCAR amplification by using the following primers: AL07, AM19, OPL19, OPB12 and Hi07h02. Specific PCR amplification of the SCAR markers was performed in an amplification reaction volume of 15 µl, including the following components in final concentration: 11,5 µl MyTaq™ Red Mix, each of the primer F and R : 0.1 µl primer (0.6 µM / µl in the final reaction volume), 3 µl DNA (10 ng / µl) and 0,3 µl ultrapure water for OPL19, respectively AD13 markers; 11,5 µl MyTaq™ Red Mix, each of the primer F and R (0,1 µl AL07, 0,1 µl AM19, 0,1 µl *Vfc*), 3 µl DNA (10 ng / µl) for PCR multiplexing; 11,5 µl MyTaq™ Red Mix, each of the primer F and R (0,1 µl OPB12 and 0,1 µl Hi07h02), 3 µl DNA (10 ng /

µl) for PCR multiplexing. Amplifications were performed in a PCR analyzer FastGene at the following conditions: AL07, AM19 and *Vfc* markers (initial denaturation step at 95°C for 1 min., followed by 35 cycles of 1 min. at 94°C, 1 min. at 60°C, 2 min. at 72°C and final extension 10 min. at 72°C); AD13 and OPL19 markers (initial denaturation step at 94°C for 2.45 min., followed by 40 cycles of 55 sec. at 94°C, 55 sec. at 58°C, 1.39 min. at 72°C, and final extension 10 min. at 72°C); OPB12 and Hi07h02 markers (initial denaturation step at 94°C for 2.45 min., followed by 40 cycles of 55 sec. at 94°C, 55 sec. at 55°C, 1.39 min. at 72°C, and final extension 10 min. at 72°C) (Table 2).

3. Results and discussions

The results of molecular screening of foreign and Romanian varieties used in Romanian breeding programmes are presented in Table 4 and Figures 1-4.

The *Rvi15* (*Vr2*) gene was mapped to the L2 linkage group by Patocchi et al. (2004) according to Liebhard et al. (2002) using an SSR marker (CH02c02a) and two AFLP markers (EA35MA41262, EA37MA39188). The OPL19SCAR marker product originally identified for the *Vh2* gene in a 'Royal Gala' × 'TSR34T15' family (Bus et al., 2000, 2005) was also linked to the *Vh8* gene in the 'Royal Gala' × 'W193B' family. The completely identical sequences of the 433 bp fragments confirmed that they are alleles of the same OPL19 SCAR marker locus and that both scab resistance genes are alleles of the same locus, closely linked loci, or homologous loci. *Vh8* is therefore located on LG2, as is the *Vh2* gene (Hemmat et al., 2002; Bus et al., 2005; Bus et al., 2005b).

In that study, using the OPL19 marker, we obtained the 433 bp allele corresponding to the *Vr* gene (*Vr2*+*Vr8*) in 10 cultivars (Figure 2), in agreement with the results published by Bus et al. (2005b) for *Vr8*, respectively Liebhard et al. (2002) and Bus et al. (2005a) for *Rvi2*.

The *Vf* gene was evidenced using three SCAR markers (AL07, AM19, *Vfc*) in a multiplex PCR reaction, when we obtained the amplification fragments associated with the gene: 570 bp with the AL07 marker, 525 bp with the AM19 marker and 286 bp with the *Vfc* marker (Fig. 1).

Similar results are also available in molecular studies published in specialized works from Romania and abroad (Gianfranceschi et al, 1996; Tartarini et al., 1999; Pătrașcu, 2006; Bodea, 2007, 2008; Militaru et al., 2020; Bivolariu et al., 2021).

Also five varieties were identified as carriers of the *Vr1* gene, and in none of the varieties included in this study did the OPB12 and Hi07h02 markers segregate with the *Rvi5* gene (Fig. 4). By using the AD13 marker, two fragments of 950 and 1200 bp corresponding to the dominant allele and the recessive allele were obtained (Fig. 3).

In the study conducted by Boudichevskaia et al. (2006) in three different populations ('Regia' × 'Pingo', 'Regia' × 'Piflora', 'Regia' × 'Piflora'), it was shown that the resistance gene, *Vr1*, is heterozygous and is present only in the resistant parent. The additional alleles detected with the AD13 SCAR primers in a small set of accessions of only a few *Malus* species are highly informative concerning molecular analyses of relationships between *Malus* genotypes as well as for taxonomic studies. To our knowledge, for this application, the AD13 locus seems to be the most informative SCAR published so far for *Malus*.

There was a tendency that plants carrying only the AD13-SCAR (class 3) or this marker in combination with the AL07-SCAR (class 1) to have a satisfactory degree of scab resistance. Considering only the *Vf* gene, the resistance data of the plants with the presence of the AL07 marker (classes 1 and 2) and the absence of this marker (classes 3 and 4), respectively, indicate complete independence between the *Vf* marker and the resistance phenotype (Boudichevskaia et al., 2006). As Rousselle et al. (1974) pointed out, the degree of resistance conferred by a major resistance gene can be modified by the action of minor or modifier genes inherited from both parents (Gygax et al., 2004).

Considering also what Mundt (2018) said who believes that: “even with highly efficient resistance genes, pyramids can sometimes be identified phenotypically", few resistance genes give a true immune response, and in many cases, combinations of resistance genes result in the reduced phenotypic expression of a pyramid compared to genotypes with fewer resistance genes", we can better understand the "genotype-phenotype-incongruence" (GPI) behaviour of plants (Gygax et al., 2004 and Erdin et al., 2006).

In 2020, at the Research Institute for Fruit Growing Pitesti - Maracineni, in a molecular screening performed with the U1400 marker, associated with the *Rvi6* gene, amplified fragments were present also in some varieties known to be susceptible to scab and in which ALO7, AM19, *Vfc* markers did not produce amplifications of the allele *Vf* associated to resistance.

4. Conclusions

Most genotypes, except 'Florina', 'Prima', 'Romus 3', 'Romus 4', 'Romus 5', 'Pionier' are susceptible to scab. The results obtained with the six molecular markers are in agreement with the phenotypic

characterisation of scab, except the 'Starkrimson' variety, although a carrier of the *Vr* and *Vr1* genes, is susceptible to scab, and may be included in the GPI (genotype-phenotype incongruence) plant category. One explanation for the incongruence between phenotypic expression and molecular characterization would be not only the influences of modifying genes but also the complexity of the gene locus. ALO7, AM19 and Vfc markers are quite suitable for marker-assisted selection in apple breeding program considering the identification of the same number of cultivars for the three markers.

In a paper published by Calenge he had said: „At the molecular level, it is clear that some QTL share structural and functional similarities with R genes. It has been demonstrated that genes sharing a common structure with R-genes could trigger a weak resistance. So if we consider the locus of the *Vr* and *Vr1* genes as a QTL trait, then we can understand the weak resistance expressed by these genes.

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Tables and Figures

Table 1. Apple varieties introduced in the study

No.	Cultivar	Genitors	Phenotypic characterization of scab
1	Romus 2	unknown	resistance
2	Romus 3	unknown	resistance
3	Romus 4	Romus 3 x Prima	resistance
4	Romus 5	Romus 3 x Prima	resistance
5	Jonagold	Jonathan x Golden Delicious	susceptible
6	Jonathan	unknown	susceptible
7	Prima	PRI 14-510 x NJ 123249	resistance
8	Wagner Premiat	unknown	susceptible
9	Golden Delicious	unknown	susceptible
10	Rome Beauty	unknown	susceptible
11	Golden Spur	spur mutation from Golden Delicious	susceptible
12	Sir Prize	Golden Delicious x PRI 14-152	moderate resistance
13	Starkrimson	Starking Delicious mutation	susceptible
14	McIntosh	unknown	susceptible
15	Granny Smith	unknown	susceptible
16	Belle de Boskoop	unknown	susceptible
17	Parmen d'or	unknown	susceptible
18	Mutsu	Golden Delicious x Indo	susceptible
19	Champion	Golden Delicious x Cox Orange Pippin	susceptible
20	Frumos de Voineşti	Jonathan x Belle de Boskoop	susceptible
21	Pionier	(Jonathan x Verzişoare) x Prima	resistance
22	Verzişoare	local variety	susceptible
23	Creţesc	Local variety	high susceptible
24	Florina	Golden Delicious x (Rome Beauty x *Malus floribunda 821*) x Starking Simpson's Giant Limb x Jonathan	resistance
25	*Malus floribunda*		resistance
26	*Malus kaido*		resistance

Table 2. Optimized amplification protocols for SSR type markers

SSR		
AL07;AM19;*Vfc*		
Initial Denaturation	1 min la 94°C	
Denaturation	**1 min la 94° C**	**35 x**
Annealing	**1 min la 58° C**	
Extending	**2 min la 72° C**	
Final Extending	10 min la 72° C	
OPL19; AD13;OPB12; Hi07h02		
Initial Denaturation	2.5 min la 94°C	
Denaturation	**55 s la 94° C**	**40 x**
Annealing	**55 s la 58° C**	
Extending	**1.39 min la 72° C**	
Final Extending	10 min la 72° C	

Table 3. Primers used for amplification of scab and powdery mildew resistance genes

Gene	Name / type marker	Primer sequence (5'→3')	Fragment size (bp)	References
Rvi6 (Vf)	AL07 / SCAR	F: TGGAAGAGAGATCCAGAAAGTG R: CATCCCTCCACAAATGCC	570; 823	Khajuria et al., 2014 Tartarini et al., 1999
Rvi6 (Vf)	AM19 / SCAR	F: CGTAGAACGGAATTTGACAGTG R: GACAAAGGGCTTAAGTGCTCC	526	Khajuria et al., 2014 Tartarini et al., 1999
Rvi6 (Vf)	*VfC* / SCAR	F: GGTTTCCAAAGTCCAATTCC R: CGTTAGCATTTTGACTTGAC	286; 484; 646	Afumian et al., 2004
Rvi4 (Vr1,Vh4, Vx)	AD13 / SCAR	F: GGTTCCTCTGTAAAGCTAG R: GGTTCCTCTGCCCAACAA	950; 1200	Boudichevskaia et al., 2006
Rvi2 (Vh2) *Rvi8 (Vh8)*	OPL19 / SCAR	F: ACCTGCACTACAATCTTCACTAATC R: GACTCGTTTCCACTGAGGATATTTG	433; 1200	Bus et al., 2005 a
Rvi5 (Vm)	OPB12 / STS	F: CCTTGACGCAGCTT R: CCTTGACGCATCTACG	687	Cheng et al., 1998
	Hi07h02		230	Patocchi et al., 2009

Table 4. Results of the molecular screening of apple cultivars used in Romanian breeding program for scab resistance using molecular markers

Cultivar	*Rvi2*	*Rvi4*	*Rvi5*	*Rvi6*			*Rvi8*	Genetic profile
	OPL19	AD13	OPB12	AL07	AM19	*VfC*	OPL19	
Remus 2	+	-	-	-	-	-	+	*Rvi2+Rvi8*
Romus 3	+	+	-	+	+	+	+	*Rvi2+Rvi8+Rvi6 +Rvi4*
Romus 4	+	-	-	+	+	+	+	*Rvi2+Rvi8+Rvi6*
Romus 5	+	-	-	+	+	+	+	*Rvi2+Rvi8+Rvi6*
Jonagold	-	-	-	-	-	-	-	
Jonathan	-	-	-	-	-	-	-	
Prima	+	-	-	+	+	+	+	*Rvi2+Rvi8+Rvi6*
Wagner Premiat	-	-	-	-	-	-	-	
Golden Delicious	-	-	-	-	-	-	-	
Rome Beauty	-	+	-	-	-	-	-	*Rvi4*
Golden Spur	-	-	-	-	-	-	-	
Sir Prize	-	-	-	+	+	+	-	*Rvi6*
Starkrimson	+	+	-	-	-	-	+	*Rvi2+Rvi8+Rvi4*
McIntosh	-	-	-	-	-	-	-	
Grany Smith	-	-	-	-	-	-	-	
Belle de Boskoop	-	+	-	-	-	-	-	*Rvi4*
Parmen d'or	+	-	-	-	-	-	+	*Rvi2+Rvi8*
Mutsu	-	-	-	-	-	-	-	
Champion	-	-	-	-	-	-	-	
Frumos de Voineşti	-	-	-	-	-	-	-	
Pionier	+	-	-	+	+	+	+	*Rvi2+Rvi8+Rvi6*
Verzişoare	-	-	-	-	-	-	-	
Creţesc	+	-	-	-	-	-	+	*Rvi2+Rvi8*
Florina	+	+	-	+	+	+	+	*Rvi2+Rvi8+Rvi6 +Rvi4*
M. floribunda	-	-	-	+	+	+	-	*Rvi6*
M. kaido	-	-	-	-	-	-	-	

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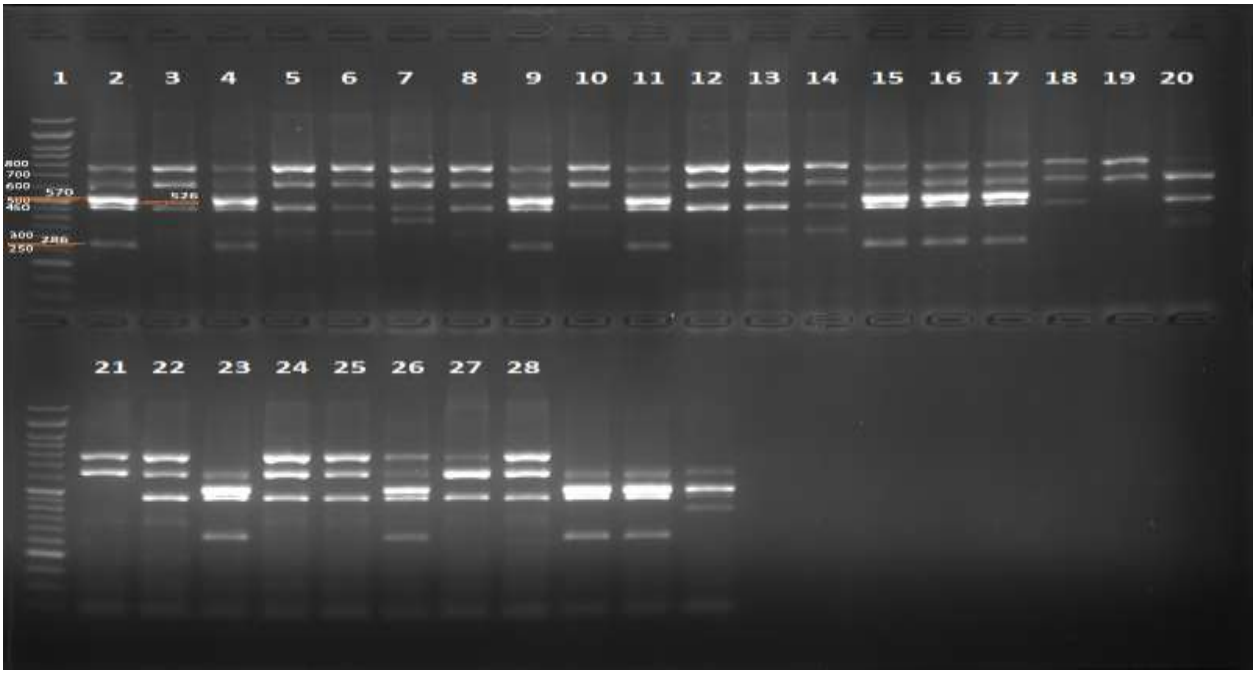

Fig. 1.The electrophoretic profile obtained with AL07, AM19 and *Vfc* markers

1. Ladder, 2. Pozitive control, 3. 'Jonathan', 4. 'Prima', 5. 'Granny Smith', 6. 'Wagner Premiat', 7. 'Belle de Boskoop', 8. 'Golden Delicious', 9. 'Florina', 10. 'McIntosh', 11. 'Pionier', 12. 'Champion', 13. 'Frumos de Voineşti', 14. 'Romus 2', 15. 'Romus 3', 16. 'Romus 4', 17. 'Romus 5', 18. 'Verzişoare', 19. 'Parmen d'or', 20. 'Creţesc', 21. 'Rome Beauty', 22. 'Frumos de Voineşti', 23.*M. floribunda*, 24. 'Golden Spur', 25. 'Mutsu', 26. 'Sir Prize', 27. *M. kaido*, 28. 'Starkrimson'

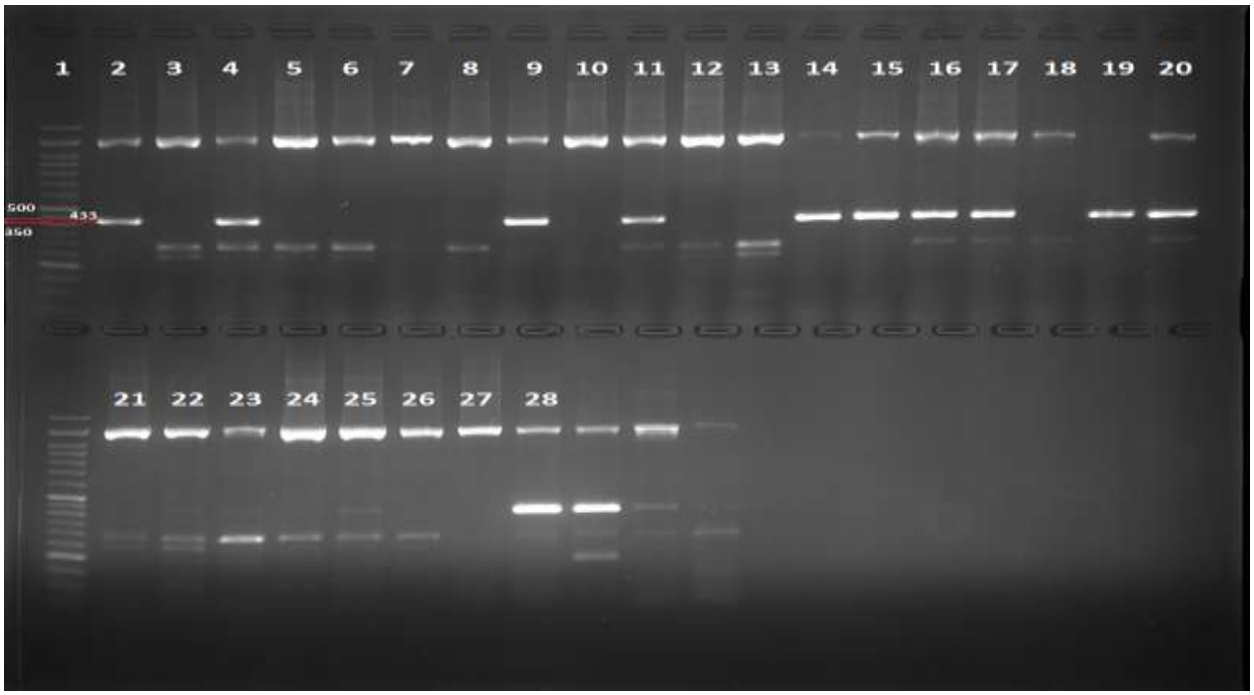

Fig. 2. The electrophoretic profile obtained with OPL19 marker

1. Ladder, 2. Pozitive control, 3. 'Jonathan', 4. 'Prima', 5. 'Granny Smith', 6. 'Wagner Premiat', 7. 'Belle de Boskoop', 8. 'Golden Delicious', 9. 'Florina', 10. 'McIntosh', 11. 'Pionier', 12. 'Champion', 13. 'Frumos de Voineşti', 14. 'Romus 2', 15. 'Romus 3', 16. 'Romus 4', 17. 'Romus 5', 18. 'Verzişoare', 19. 'Parmen d'or', 20. 'Creţesc', 21. 'Rome Beauty', 22. 'Frumos de Voineşti', 23.*M. floribunda*, 24. 'Golden Spur', 25. 'Mutsu', 26. 'Sir Prize', 27. *M. kaido*, 28. 'Starkrimson'

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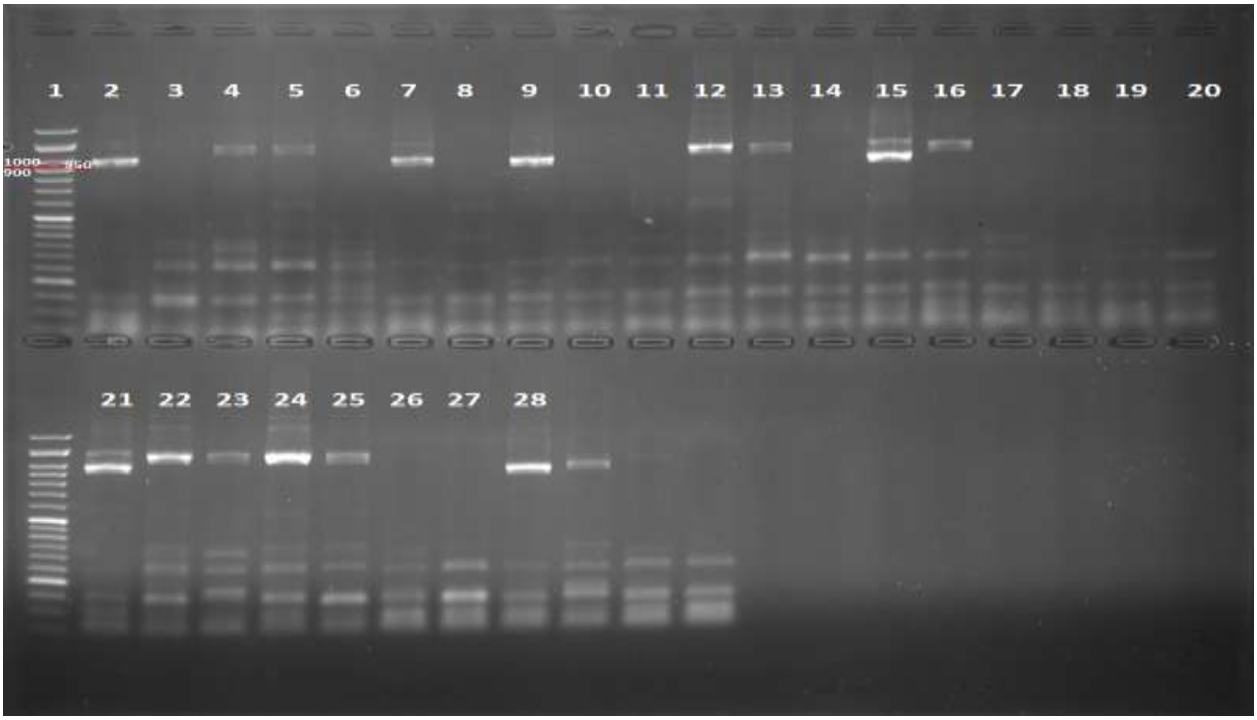

Fig. 3. The electrophoretic profile obtained with AD13 marker
1. Ladder, 2. Pozitive control, 3. 'Jonathan', 4. 'Prima', 5. 'Granny Smith', 6. 'Wagner Premiat', 7. 'Belle de Boskoop', 8. 'Golden Delicious', 9. 'Florina', 10. 'McIntosh', 11. 'Pionier', 12. 'Champion', 13. 'Frumos de Voineşti', 14. 'Romus 2', 15. 'Romus 3', 16. 'Romus 4', 17. 'Romus 5', 18. 'Verzişoare', 19. 'Parmen d'or', 20. 'Creţesc', 21. 'Rome Beauty', 22. 'Frumos de Voineşti', 23.*M. floribunda*, 24. 'Golden Spur', 25. 'Mutsu', 26. 'Sir Prize', 27. *M. kaido*, 28. 'Starkrimson'

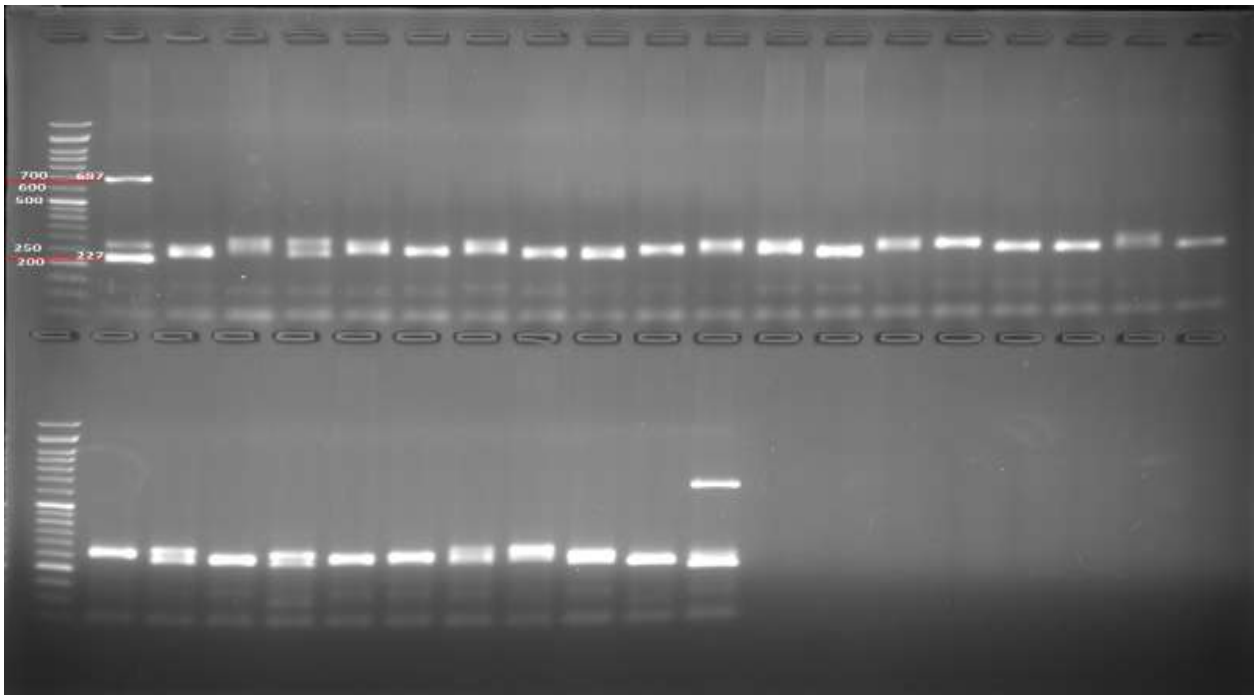

Fig. 4. The electrophoretic profile obtained with OPB12 and Hi07h02 marker
1. Ladder, 2. Pozitive control, 3. 'Jonathan', 4. 'Prima', 5. 'Granny Smith', 6. 'Wagner Premiat', 7. 'Belle de Boskoop', 8. 'Golden Delicious', 9. 'Florina', 10. 'McIntosh', 11. 'Pionier', 12. 'Champion', 13. 'Frumos de Voineşti', 14. 'Romus 2', 15. 'Romus 3', 16. 'Romus 4', 17. 'Romus 5', 18. 'Verzişoare', 19. 'Parmen d'or', 20. 'Creţesc', 21. 'Rome Beauty', 22. 'Frumos de Voineşti', 23.*M. floribunda*, 24. 'Golden Spur', 25. 'Mutsu', 26. 'Sir Prize', 27. *M. kaido*, 28. 'Starkrimson'