Fruit Growing Research, Vol. XXXVIII, 2022 DOI 10.33045/fgr.v38.2022.09
https://publications.icdp.ro/index.php

CARACTERIZAREA MORFOLOGICĂ ŞI A DIVERSITĂŢII GENETICE PRIN UTILZAREA TEHNICII SSR LA UNELE GENOTIPURI DE MĂR

MORPHOLOGICAL CHARACTERIZATION AND GENETIC DIVERSITY USING THE SSR TECHNIQUE IN SOME APPLE GENOTYPES

Iancu Adina, Chivu Mihai
Research Institute for Fruit Growing Pitesti, Romania

Abstract

The scab is a widespread disease throughout the world causing large financial losses in apple production and needs to be controlled also by developing polygenic varieties with resistance to several races of the pathogen *Venturia inaequalis* by incorporation of two or more functionally different resistance genes. The studies for evidence of interest genes relating to the resistance of apple varieties to apple scab have been conducted using several types of molecular markers (SCAR, SSR, RAPD, ISSR etc.). In this experiment, using six SSR molecular markers (CH02b10, CH05e03, CH02d01, Hi07f01 and Hi07h02), was tracked the reveal of amplified fragments, corresponding to PCR products associated with resistance genes *Rvi2*, *Rvi8*, *Rvi5* and *Rvi11*, but also the intraspecific diversity expressed at the molecular level of Romanian apple varieties, some of them having common genitors. The position of the amplified fragments on the agarose gel for the six SSR markers was located on similar values ranges to those published in various specialized papers, the size of the amplified fragments following to be evaluated by the sequencing step and published in a new paper as an addition to the results of this study.

Cuvinte cheie: măr, diversitate genetică, *Rvi2*, *Rvi8*, *Rvi11*, tehnica SSR, gene de rezistenţă.
Key words: apple, genetic diversity, *Rvi2*, *Rvi8*, *Rvi11*, SSR technique, resistance genes.

1. Introduction

Analysis of genotypes at the molecular level using different molecular fingerprinting techniques for genes of interest has the aim to make more efficient use of germplasm for breeding purposes. Breeding for scab-resistant apple cultivars by pyramiding several resistance genes in the same genetic background is a promising way to control apple scab caused by the fungus *Venturia inaequalis* (Gygax at al., 2004). Obtaining varieties with durable resistance to scab, with quality fruit in view of organoleptic properties and a long storage period are major objectives included in apple breeding programmes. SSR type molecular marker assisted selection is successfully used for mapping genes of interest, in the study such us: for *Rvi15* gene (Galli et. al., 2010), for *Rvi2* and *Rvi4* gene (Bus et. al., 2005a), *Rvi11* (Gygax et al., 2004). Various molecular markers have been used for the evidence of scab resistance genes such as: for the *Rvi2* gene (Ch02b10, Ch05e03 and CH03d01) in studies realized by: Bus et al. (2005a), Höfer et al. (2021), Patocchi et al. (2009), Hemmat et al. (2002) (Table 4); for the genes *Rvi8* and *Rvi11* (CH03d01) in studies realized by: Gygax et al. (2004), Bus et al. (2005b), Höfer et al. (2021), Patocchi et al., 2009 (Table 4) and, respectively, for the *Rvi5* gene (Hi07h02) in studies by Patocchi et al. (2005), Patocchi et al., (2009), Cova et al. (2015) (Table 4). SSR type markers are also very useful in identifying polymorphism at the varieties grown in different regions, as can be seen from finished studies of varieties: 'Florina', 'Prima', 'Idared', 'Golden Delicious', 'Jonagold', 'Granny Smith', respectively for some accessions: 'TSR34T15' and 'TSR33T239', considered differential hosts for genes *Rvi2* şi *Rvi4* (Table 4).

2. Material and methods

Molecular analyses for DNA isolation and purification were performed according to the extraction protocol recommended by de "ISOLATE II Plant DNA Kit", Bioline and PCR-SSR amplification by using the following primers: CH02b10; CH05e03; Ch02c06; CH03d01; Hi07f02 and Hi07h02. The DNA amplification reaction, for each sample, was optimized in a volume of 15 µl, including the following components in final concentration: 11.7 µl MyTaq™ Red Mix, 0,3 µl primer (0.6 µM / µl in the final reaction volume), 3 µl DNA (10 ng / µl). The samples thus prepared were worked the following amplification programmes: initial denaturation at 94ºC (3 mins); followed by 35 cycles: 94ºC (1 min), 58ºC (1 min), 72ºC (2 mins) and a final extension of 10 mins at 72ºC for CH02b10; initial denaturation at 94ºC (4 min); followed by 45 cycles: 94ºC (50 s), 55ºC (50 s), 72ºC (50 s) and a final 5 min extension at 72ºC for CH05e03 (Table 2).

Verification of the resulting amplicons from the polymerase chain reaction PCR was realized after run the samples through the agarose gel into a horizontal electrophoresis system, using a transilluminator Essential V6 by capturing the image on the gel (Fig. 1, 2, 3, 4, 5).

3. Results and discussions

The results of the molecular screening effectuated for 22 apple varieties at RIFG Pitesti, Romania (Militaru et al., 2020, 2022) revealed the Vr gene (*Rvi2*+*Rvi8)* in 13 varieties by segregating the OPL19 marker with the locus of the two genes, 8 of which were also included in this study (Table 1). Therefore, for these 8 varieties ('Romus 3', 'Romus 5', 'Verzișoare', 'Delicios de Voinești', 'Remar', 'Aura', 'Domnesc' and 'Crețesc') we should be able to evidence the PCR product associated to the resistance genes *Rvi2* and *Rvi8* using microsatellites CH02b10, CH05e03 and CH03d01. Analysis of the electrophoretic profile allowed verification of the length of PCR product. The appreciation of these values from images obtained with the software "UVitec v. 1.0." was possible using the software "Image Lab v. 6.1.", Biorad. The sizes of the base pairs associated with the amplified fragments in our study are contained in ranges of values similar to those obtained in various literature (Table 3).

So far we have completed the PCR amplification step for all 6 markers and optimized the working protocol for vertical electrophoresis, In the future, the next step will be focus on the sequencing to be able to associate the amplified fragments with the genes of interest, where the markers used segregated with the gene locus and the PCR products were different from literature, but also to observe differences in the genome of a variety grown in different regions. The *Rvi2* and *Rvi8* scab-resistance genes are alleles of the same locus, closely linked loci, or homologous loci (Bus et. al., 2005b).

4. Conclusions

Confirming the presence of *Rvi2* and *Rvi8* genes using several molecular analysis techniques is evidence of use in breeding programmes for those parents with a high potential for the inheritance of dominant traits of interest. On the other hand, the *Vr* gene (*Rvi2*+*Rvi8*) provides resistance only to races 1,2,3,4,5,6,7,8 of the pathogen *Venturia inaequalis*, and Romanian apple varieties presenting only the *Vr* gene, some of them also introduced in our study ('Verzișoare', 'Delicios de Voinești', 'Domnesc', 'Crețesc'), are susceptible to scab. Therefore, in Romania, other breeds of this pathogen are present besides those 8. Breeding programmes are challenged to achieve controlled hybridization between suitable parents for the evolution towards sustainable agriculture.

Acknowledgements

This research was partially supported by the Romanian Ministry of Agriculture and Rural Development, project ADER 7.2.6/2021.

References

1. Akpınar A.E., Altıntaș S, Altunok S, Akçay M.E., Karataș M.D., Ergül A., 2018. Genetic characterization of some apple (*Malus*× *domestica* Borkh.) Genotypes based on SSRs (Simple Sequence Repeats). Gaziosmanpașa Üniversitesi Ziraat Fakültesi Dergisi, 35 (3): 268-277.
2. Boudichevskaia A., Dunemann F., Stankiewicz-Kosyl M., Mathis-Jeanneteau F., Durel C., Gianfranceschi L., Costa F., Toller C., Cova V., Mott D., Komjanc M., Barbaro E., Kodde L., Rikkerink E., Gessler C., van de Weg W., 2009. Development and test of 21 multiplex PCRs composed of SSRs spanning most of the apple genome. Tree Genetics & Genomes, 5(1): 211-223.
3. Bus V.G.M., Laurens F.N.D, van de Weg W.E, Rusholme RL, Rikkerink E.H.A, 2005b. The Vh8 locus of a new gene-for-gene interaction between Venturia inaequalis and the wild apple *Malus sieversii* is closely linked to the Vh2 locus in *Malus pumila* R12740–7A. New Phytol. 166: 1035–49.
4. Bus V.G.M., Rikkerink E.H.A., van de Weg W.E., Rusholme R.L., Gardiner S.E., Bassett H.C.M. et. al., 2005a. The Vh2 and Vh4 scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. Molecular Breeding, 15(1): 103-116.
5. Cova V., Bandara N.L., Liang W., Tartarini S., Patocchi A., 2015. Fine mapping of the Rvi5 (Vm) apple scab resistance locus in the 'Murray' apple genotype. Molecular Breeding, 35(10): 200.
6. Dayton D.F. and Williams, E.B. 1970. Additional allelic genes in *Malus* for scab resistance of two reaction types. J. Am. Soc. Hortic. Sci., 95: 735–736.
7. Galli P., Broggini G.A.L., Kellerhals M., Gessler C., Patocchi A., 2010. High-resolution genetic map of the Rvi15 (Vr2) apple scab resistance locus. Molecular Breeding 26(4): 561-572.

8. Galli Z., Halász G., Kiss E., Heszky L., Dobránszki J., 2005. Molecular identification of commercial apple cultivars with microsatellite markers. HortScience, 40: 1974–1977.
9. Gasi F., Simon S., Pojskic N., Kurtovic M., Pejic I., 2010. Genetic assessment of apple germplasm in Bosnia and Herzegovina using microsatellite and morphologic markers. Sci. Hortic. 126: 164–171.
10. Gygax M., Gianfranceschi L., Liebhard R., Kellerhals M., Gessler C., Patocchi A. 2004. Molecular markers linked to the apple scab resistance gene Vbj derived from *Malus baccata jackii*. Theoretical and Applied Genetics, 109(8): 1702-1709. doi:10.1007/s00122-004-1803-9.
11. Hemmat M., Brown S.K. and Weeden N.F. 2002. Tagging and mapping scab resistance genes from R12740–7A apple. J. Am. Soc. Hortic. Sci. 127: 365–370.
12. Hemmat M., Weeden N., Susan K., Brown, 2003b. Mapping and Evaluation of *Malus* × *domestica* Microsatellites in Apple and Pear. Journal of the American Society for Horticultural Science, 128(4): 515-520.
13. Höfer M., Flachowsky H., Schröpfer S., Peil A. 2021. Evaluation of scab and mildew resistance in the Gene Bank collection of apples in Dresden-Pillnitz. Plants, 10 (6): 1227.
14. Khankishiyeva E., 2020. Screening for resistance against venturia inaequalis (cke.) wint and Podosphaera leucotricha in introduced varieties of apple in azerbaijan, using molecular markers. Research in: Agricultural & Veterinary Sciences, 4(3): 104-115.
15. Liebhard R., Gianfranceschi L., Koller B., Ryder C.D., Tarchini R., Van de Weg E., Gessler C., 2002. Development and characterisation of 140 new microsatellites in apple (*Malus* × *domestica* Borkh.). Molecular Breeding 10: 217–241.
16. Liebhard R., Koller B., Patocchi A., Kellerhals M., Pfammatter W., Jermini M. and Gessler C. 2002. Mapping quantitative field resistance against apple scab in a Fiesta x Discovery progeny. Phytopathology 93: 493–501.
17. Liebhard R., 2003. Genetic dissection of physiological traits and scab resistance in apple (*Malus x domestica* (Borkh.)) into quantitative trait loci using an SSR based genetic linkage map. Doctoral Thesis. https://doi.org/10.3929/ethz-a-004552369.
18. Maliepaard C., Alston F., Arkel G., Brown L., Chevreau E., Dunemann F., Evans K., Gardiner S., Guilford P., Heusden S., Janse J., Laurens F., Lynn J., Manganaris S., Nijs T., Periam N., Rikkerink E., Roche P., Ryder C., Sansavini S., Schmidt H., Tartarini S., Verhaegh J., Vrielink R., King G., 1998. The european apple map. Acta Hortic. 56: 484.
19. Marconi G., Ferradini N., Russi L., Concezzi L., Veronesi F., Albertini E., 2018. Genetic characterization of the apple Germplasm collection in Central Italy: the value of local varieties, Front Plant Sci, 9:1460: 1-17.
20. Meland M., Aksic M.F., Frøynes O., Konjic A., Lasic L., Pojskic N., Gasi F., 2022. Genetic Identity and Diversity of Apple Accessions within a Candidate Collection for the Norwegian National Clonal Germplasm Repository. Horticulturae, 8: 630.
21. Patocchi A., Frei A., Frey J.E., Kellerhals M., 2009. Towards improvement of marker assisted selection of apple scab resistant cultivars: *Venturia inaequalis* virulence surveys and standardization of molecular marker alleles associated with resistance genes. Mol. Breed.24: 337–347.
22. Patocchi A., Walser M., Tartarini S., Broggini G.A., Gennari F., Sansavini S. et al., 2005. Identification by genome scanning approach (GSA) of a microsatellite tightly associated with the apple scab resistance gene Vm. Genome, 48(4): 630-636. 2
3. Patzak Josef, Paprstein František and Henychova Alena, 2011. Identification of apple scab and powdery mildew resistance genes in Czech apple (*Malus* × *domestica*) genetic resources by PCR molecular markers. Plant Breed, 47: 156–165.
24. Pop R., Hâța M, Szabo K., Zănescu M., Sisea C.R. , Catană C., Pamfil D., 2017. Genetic Diversity and Population Structure of Plum Accessions from a Romanian Germplasm Collection Assessed by Simple Sequence Repeat (SSR) Markers. Notulae Botanicae Horti Agrobotanici, Cluj-Napoca, 46(1): 90-96.
25. Scot H. Hulbert, et al., 2001. Resistance gene complexes: Evolution and Utilization. Annual Review of Phytopathology 39(1): 285-312.
26. Sikorskaite S., Gelvonauskiene D., Stanys V., Baniulis D. 2012. Characterization of microsatellite loci in apple (Malus x domestica Borkh.) cultivars. Zemdirbyste-Agriculture, 99 (2): 131–138.
27. Silfverberg-Dilworth E., Matasci C.L., Van de Weg W.E., Van Kaauwen M.P.W., Walser M., Kodde L.P., Soglio V., Gianfranceschi L., Durel C.E., Costa F., Yamamoto T., Koller B., Gessler C., Patocchi A., 2006. Microsatellite markers spanning the apple (*Malus x domestica* Borkh.). Tree Genetics & Genomes, 2: 202–224.
28. Tania G.L., 2012. Genetic mapping and characterization of resistance factors in apple (*Malus* sp.) against fire blight. Wien, Univ. für Bodenkultur, Diss.

29. Van Treuren R, Kemp H., Ernsting G., Jongejans B., Houtman H., Visser L., 2010. Microsatellite genotyping of apple (Malus 3 domestica Borkh.) genetic resources in the Netherlands: application in collection management and variety identification. Crop Evol 57: 853-865.
30. Wichmann B., Galli Z., Molnár S., Galbács Z., Kiss E., Szabó T., 2007. Molecular identification of old Hungarian apple varieties. International Journal of Horticultural Science, 13(3): 37–42.

Tables and Figures

Table 1. Apple cultivars studied

No.	Cultivar	Genitors, origin	Phenotypic characterization of scab
1	Romus 3	Interspecific hybrid F4, Romania	resistance
2	Romus 5	Romus 3 x Prima, Romania	resistance
3	Verzişoare	Local variety, Romania	susceptible
4	Parmain auriu	Unknoun parents, England	susceptible
5	Domnesc	Local variety, Romania	susceptible
6	Creţesc	Local variety, Romania	susceptible
7	Florina	Golden Delicious x (Rome Beauty x *Malus Floribinda 82*) x Starking Simpson's Giant Limb x Jonathan, France	resistance
8	Remar	Gamma radiation(5000 ϒ) of Prima seeds (o.p.), Romania	resistance
9	Prima	PRI 14-510 x NJ 123249, USA	resistance
10	Generos	(Parmain d'or x *Malus Kaido*) x (Jonathan x V 53-39-2) x (Frumos de Voinesti x V 60-6-51), Romania	susceptible
11	Valery	Golden Spur x Florina, Romania	resistance
12	Rebra	Prima x Florina, Romania	moderate resistance
13	Rustic	Florina x Pionier, Romania	moderate resistance
14	Idared	Jonathan x Wagener Premiat, USA	susceptible
15	Pionier	Jonathan x Verzişoare x Prima, Romania	moderate resistance
16	Jonathan	Unknoun parents, USA	susceptible
17	Belle de Booskop	Unknoun parents, Netherlands	susceptible
18	Golden Delicious	Unknoun parents, USA	susceptible
19	Delicios de Voineşti	Golden Delicious x Creţesc, Romania	susceptible
20	Rome Beauty	Unknoun parents, USA	susceptible
21	Wagner Premiat	Unknoun parents, USA	susceptible
22	Jonagold	Jonathan x Golden delicious,Romania	susceptible
23	*M. floribunda*		moderate resistance
24	Granny Smith	Hibrid din *Malus sylvestris*, Australia	susceptible
25	Creţesc auriu	Local variety, Romania	susceptible
26	Aura	Prima x BN 33/39, Romania	resistance

Table 2. Optimized amplification protocols for SSR type markers

CH02b10		
Initial Denaturation	3 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	
CH05e03		
Initial Denaturation	4 min la 94°C	
Denaturation	**50 s la 94° C**	**45 x**
Annealing	**50 s la 55° C**	
Extending	**50 s la 72° C**	
Final Extending	5 min la 72° C	
CH03d01		
Initial Denaturation	2 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	
Hi07f01		
Initial Denaturation	2 min la 94°C	
Denaturation	**30 s la 94° C**	**35 x**
Annealing	**30 s la 55° C**	

Extending	**1 min la 72° C**	
Final Extending	10 min la 72° C	
CH02c06		
Initial Denaturation	2 min la 94°C	
Denaturation	**1 min la 94° C**	**40 x**
Annealing	**1 min la 55° C**	
Extending	**2 min la 72° C**	
Final Extending	10 min la 72° C	
Hi07h02		
Initial denaturation	2 min la 94°C	
Denaturation	**1 min la 94° C**	**40 x**
Annealing	**1 min la 55° C**	
Extending	**2 min la 72° C**	
Final Extending	10 min la 72° C	

Table 3. Range of values for base pairs in various molecular studies for SSR markers in the apple species

No.	Markers SSR	Nucleotide sequence	Range of values (bp)	Reference
1	**CH02b10**	F: 5'- CAAGGAAATCATCAAAGATTCAAG -3' R: 5'-CAAGTGGCTTCGGATAGTTG-3'	122	Hemmat et al., 2002
			121-159	HiDRAS site
			121-159	Gianfranceschi et al., 1998
			112–160	Sikorskaite et al., 2012
			120-150	**This study (Fig. 2)**
2	**CH05eO3**	F: 5'-CGAATATTTTCACTCTGACTGGG-3' R: 5'-CAAGTTGTTGTACTGCTCCGAC-3'	158-190	Gianfranceschi et al., 1998
			160-193	Galli et al., 2005
			153-193	Gasi et al., 2010
			145-224	Marconi et al., 2018
			158-190	Liebhard et al., 2002
			144-196	Meland et al., 2022
			149-193	Wichmann et al., 2007
			154-224	**This study (Fig. 1)**
3	**CH03d01**	F: 5'- CGCACCACAAATCCAACTC -3' R: 5'- AGAGTCAGAAGCACAGCCTC -3'	95-115	Gianfranceschi et al., 1998
			95-115	Lu et al., 2010
			94–118	Patocchi et al., 2009
			90-121	**This study**
4	**Hi07f01**	F: 5'- GGAGGGCTTTAGTTGGGAAC -3' R: 5'- GTTTGAGCTCCACTTCCAACTCC -3'	204-220	HiDRAS site
			180–218	Patocchi et al., 2009
			205-240	**This study (Fig. 4)**
5	**CH02c06**	F: 5'- TGACGAAATCCACTACTAATGCA -3' R: 5'- GATTGCGCGCTTTTTAACAT -3'	218-263	Gasi et al., 2010
			204-264	Meland et al., 2022
			216-254	Gianfranceschi et al., 1998
			201–265	Sikorskaite et al., 2012
			220-268	**This study (Fig. 3)**
6	**Hi07h02**	F: 5'- CAAATTGGCAACTGGGTCTG -3' R: 5'- GTTTAGGTGGAGGTGAAGGGATG -3'	223–279	Patocchi et al., 2009
			246-276	HiDRAS site
			238-276	Marconi et al., 2018
			223-347	**This study (Fig. 5)**

Table 4. The genetic diversity analysis using SSR and SCAR technique for scab resistance genes from the apple species in previous studies in the worldwide

Cultivar	Molecular markers	Amplified fragments (bp)	Evidenced gene	Reference
Romus 3	OPL19	**433**:1200	***Vr* (*Rvi2+Rvi8*)**	Militaru et al., 2020
Romus 5	OPL19	**433**:1200	***Vr* (*Rvi2+Rvi8*)**	Militaru et al., 2020
Domnesc	OPL19	**433**:1200	***Vr* (*Rvi2+Rvi8*)**	Militaru et al., 2020
Florina	CH05e03	163 :191		Galli et al., 2005
		162:188		Akpınar et al., 2018
		162:188		Liebhard et al., 2002
	CH02b10	133:135		Liebhard et al., 2002
	CH02c06	250		Liebhard, 2003
	CH03d01	111:113		Liebhard et al., 2002
	Hi07f01	206:220		Silfverberg-Dilworth et al., 2006

 DOI 10.33045/fgr.v38.2022.09

	Hi07h02	246:256		Silfverberg-Dilworth et al., 2006
	OPL19	**433**:1200	***Vr* (*Rvi2+Rvi8*)**	Militaru et al., 2020
Remar	OPL19	**433**:1200	***Vr* (*Rvi2+Rvi8*)**	Militaru et al., 2020
Prima	CH05e03	179:185		Galli Z. et al., 2005
		186:192		Van Treuren et al., 2010
		176:182		Liebhard et al., 2002
	Hi07f01	210:220		Silfverberg-Dilworth et al., 2006
	Hi07h02	248:274		Silfverberg-Dilworth et al., 2006
	CH02b10	123:127		Liebhard et al., 2002
		126:143		Sikorskaite, et al., 2012
	Ch02c06	236:240		Liebhard, 2003
		247:255		Sikorskaite, et al., 2012
	Ch03d01	111:113		Liebhard et al., 2002
Rome Beauty	CH02b10	120:130		Hemmat et al., 2003b
White Angel		115:160		
Idared	CH05e03	172 :185		Galli. et al, 2005
		171:183		Tania, 2012
	Hi07h02	202:202		Silfverberg-Dilworth et al., 2006
Jonathan	CH05e03	163 :185		Galli et al., 2005
Golden Delicious	CH05e03	179:185		Galli et al., 2005
		174:181		Höfer et al., 2021
		185:191		Patocchi et al., 2009
		176:182		Akpınar et. al., 2018
	CH02b10	123:126		Höfer et al., 2021
		126:130		Patocchi et al., 2009
	Hi07h02	247:255		Höfer et al., 2021
		251:259		Patocchi et al., 2009
		248:256		Patocchi et al., 2005
	CH3d01	113		Höfer et al., 2021
	CH02c06	235:239		Höfer et al., 2021
Jonagold	CH05e03	163 :179:185		Galli et al., 2005
		163:178:184		Gasi şi colab., 2010
	CH02c06	236:241:253		Gasi et al., 2010
Granny Smith	CH05e03	168:181		Galli et al., 2005
		167:180		Gasi et al., 2010
	CH02c06	230:236		Gasi et al., 2010
TSR34T15	CH05e03	**163**		Höfer et al., 2021
		165		Bus et al., 2005
	CH02b10	**122**:146	***Rvi2***	Höfer et al., 2021
		125:152		Patocchi et al., 2009
		121		Bus et al., 2005a
	Ch03d01	**119**		Bus et al., 2005a
TSR33T239	CH02c02a	**176**		Höfer et al., 2021
		183	***Rvi4***	Patocchi et al., 2009
		170		Bus et al., 2005a
Empire x R12740-7A	CH02b10	**122**		Hemmat et al., 2002
White Angel x Rome Beauty		**122**	***Rvi2***	Hemmat et al., 1994
9-AR2T196	Hi07h02	226:273	***Rvi5***	Höfer et al., 2021
		230:277		Patocchi et al., 2009
M. baccata var. jackii	CH05e03	**150**		Höfer et al., 2021
		160		Patocchi et al., 2009
	CH03d01	**103:105**		Höfer et al., 2021
	CH02c06	**233:245**	***Rvi11***	Höfer et al., 2021
A722-7 × cv. Golden Delicious *Malus baccata* jackii OR18T26 (USA)	CH05e03	**150 (176,182,190)**		Gygax et al., 2004
	CH02c06	**248 (230,236,240)**		
	CH03d01	**115 (109,113)**		
M. sieversii W193B	Ch03d01	**124**	***Rvi8***	Bus et al., 2005b
Murry x Golden Delicious	Hi07h02	**227**	***Rvi5***	Patocchi et al., 2005
		224:271		Cova et al., 2015

 DOI 10.33045/fgr.v38.2022.09

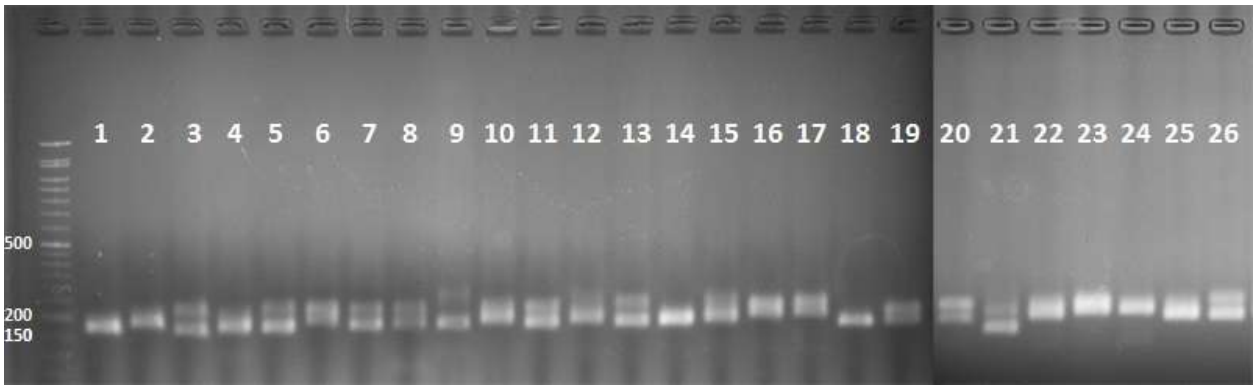

Fig. 1. Electrophoretic profile obtained with marker CH05e03 (154-224 bp)
1. 'Creţesc', 2. 'Generos', 3. 'Domnesc', 4. 'Romus 3', 5. 'Valery', 6. 'Remar', 7. 'Rebra', 8. 'Rustic', 9. 'Florina', 10. 'Idared', 11. 'Pionier', 12. 'Verzişoare', 13. 'Jonathan', 14. 'Parmen d'or', 15. 'Belle de Boskoop', 16. 'Golden Delicious', 17. 'Delicios de Voineşti', 18. 'Rome Beauty', 19. 'Wagner Premiat', 20. 'Jonagold', 21. *Malus floribunda*, 22. 'Grany Smith', 23. 'Aura', 24. 'Creţesc auriu', 25. 'Romus 5', 26. 'Prima'

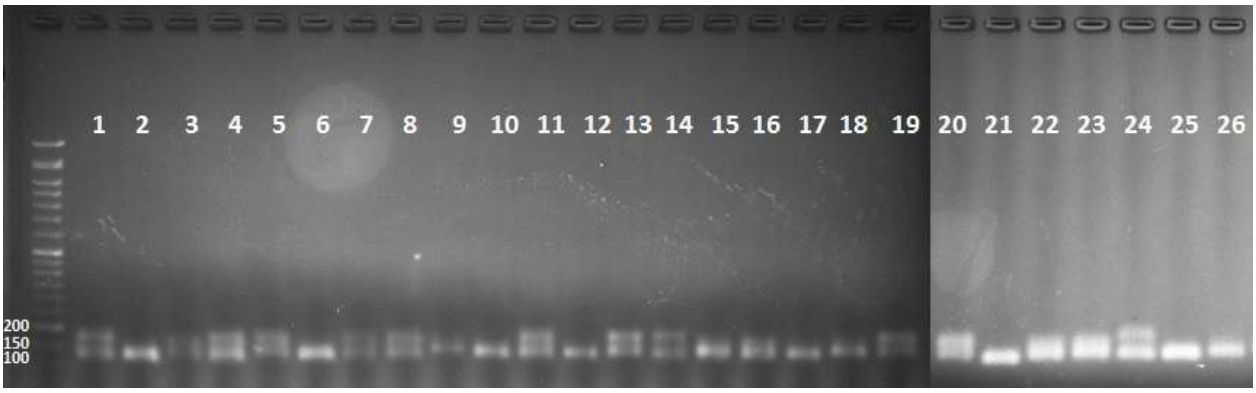

Fig. 2. Electrophoretic profile obtained with marker CH02d10 (120-172bp)
1. 'Creţesc', 2. 'Generos', 3. 'Domnesc', 4. 'Romus 3', 5. 'Valery', 6. 'Remar', 7. 'Rebra', 8. 'Rustic', 9. 'Florina', 10. 'Idared', 11. 'Pionier', 12. 'Verzişoare', 13. 'Jonathan', 14. 'Parmen d'or', 15. 'Belle de Boskoop', 16. 'Golden Delicious', 17. 'Delicios de Voineşti', 18. 'Rome Beauty', 19. 'Wagner Premiat', 20. 'Jonagold', 21. *Malus floribunda*, 22. 'Grany Smith', 23. 'Aura', 24. 'Creţesc auriu', 25. 'Romus 5', 26. 'Prima'

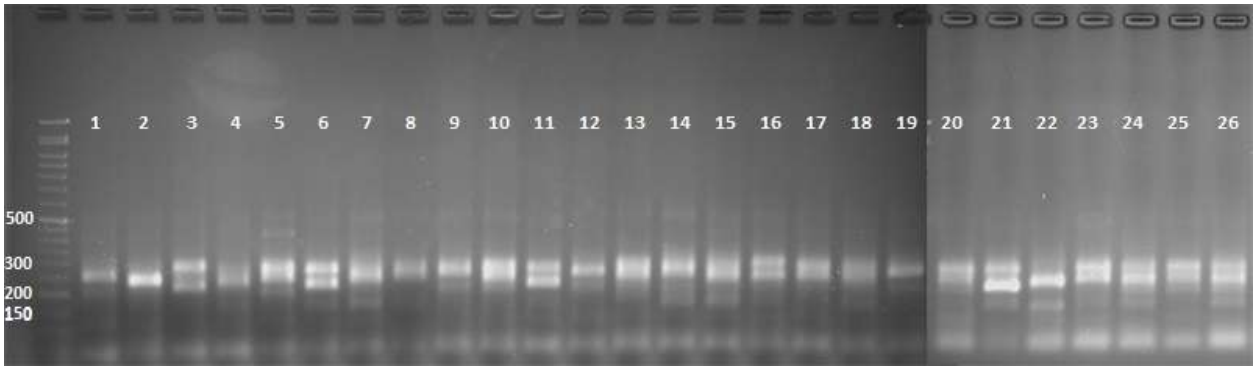

Fig. 3. Electrophoretic profile obtained with marker CH02C06 (220-268 bp)
1. 'Creţesc', 2. 'Generos', 3. 'Domnesc', 4. 'Romus 3', 5. 'Valery', 6. 'Remar', 7. 'Rebra', 8. 'Rustic', 9. 'Florina', 10. 'Idared', 11. 'Pionier', 12. 'Verzişoare', 13. 'Jonathan', 14. 'Parmen d'or', 15. 'Belle de Boskoop', 16. 'Golden Delicious', 17. 'Delicios de Voineşti', 18. 'Rome Beauty', 19. 'Wagner Premiat', 20. 'Jonagold', 21. *Malus floribunda*, 22. 'Grany Smith', 23. 'Aura', 24. 'Creţesc auriu', 25. 'Romus 5', 26. 'Prima'

 DOI 10.33045/fgr.v38.2022.09

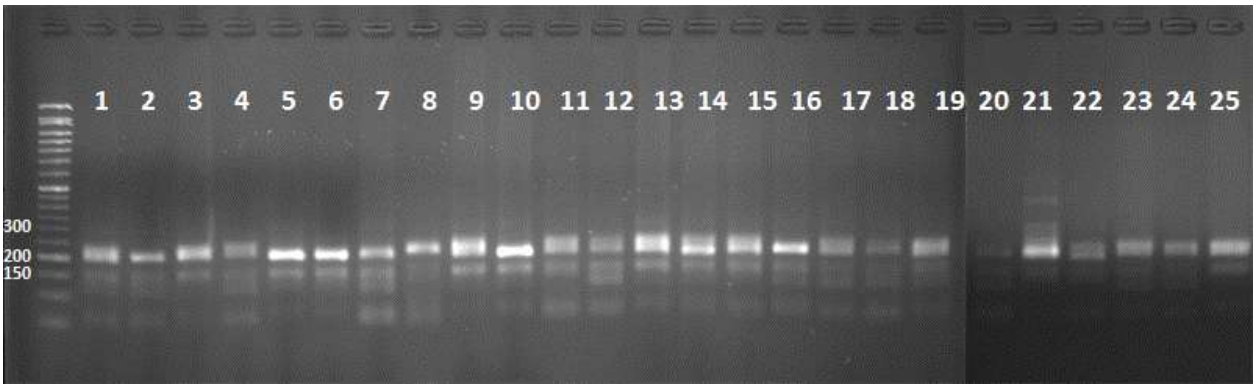

Fig. 4. Electrophoretic profile obtained with marker Hi07f01 (205-240 bp)
1. 'Creţesc', 2. 'Generos', 3. 'Domnesc', 4. 'Romus 3', 5. 'Valery', 6. 'Remar', 7. 'Rebra', 8. 'Rustic', 9. 'Florina', 10. 'Idared', 11. 'Pionier', 12. 'Verzişoare', 13. 'Jonathan', 14. 'Parmen d'or', 15. 'Belle de Boskoop', 16. 'Golden Delicious', 17. 'Delicios de Voineşti', 18. 'Rome Beauty', 19. 'Wagner Premiat', 20. 'Jonagold', 21. *Malus floribunda*, 22. 'Grany Smith', 23. 'Aura', 24. 'Creţesc auriu', 25. 'Romus 5'

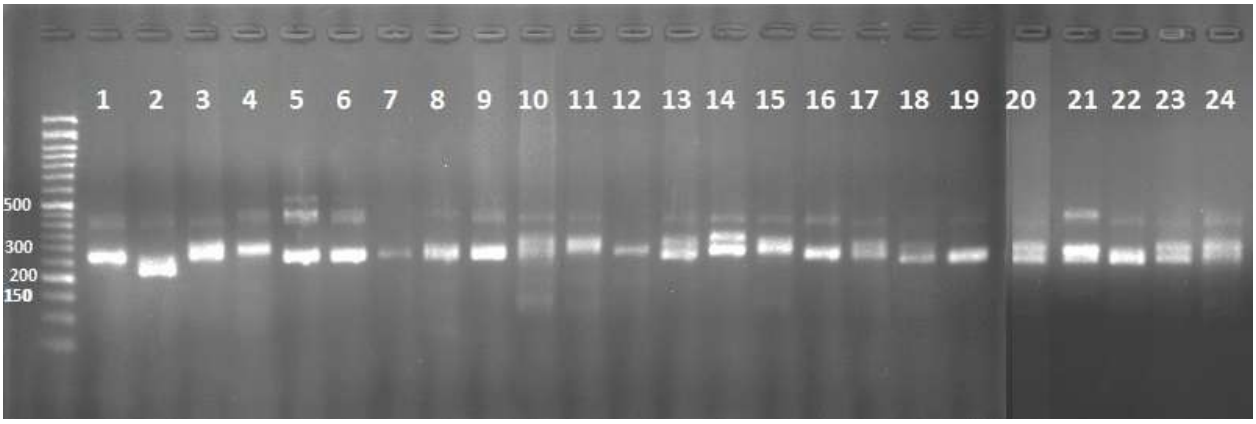

Fig. 5. Electrophoretic profile obtained with marker Hi07h02 (223-347bp)
1. 'Creţesc', 2. 'Generos', 3. 'Domnesc', 4. 'Romus 3', 5. 'Valery', 6. 'Remar', 7. 'Rebra', 8. 'Rustic', 9. 'Florina', 10. 'Idared', 11. 'Pionier', 12. 'Verzişoare', 13. 'Jonathan', 14. 'Parmen d'or', 15. 'Belle de Boskoop', 16. 'Golden Delicious', 17. 'Delicios de Voineşti', 18. 'Rome Beauty', 19. 'Wagner Premiat', 20. 'Jonagold', 21. *Malus floribunda*, 22. 'Grany Smith', 23. 'Aura', 24. 'Creţesc auriu'