Research in: Agricultural & Veterinary Sciences
Vol.4, No.3, 2020, pp.104-115

SCREENING FOR RESISTANCE AGAINST *VENTURIA INAEQUALIS* (CKE.) WINT AND *PODOSPHAERA LEUCOTRICHA* IN INTRODUCED VARIETIES OF APPLE IN AZERBAIJAN, USING MOLECULAR MARKERS

Elnara Khankishiyeva*

Scientific Research Institute of Fruit- and Tea-growing, Ministry of Agriculture,
Guba, Azerbaijan

Abstract. In this study, using molecular approaches, we evaluated the resistance to the pathogen *Venturia inaequalis* (Cke.) of some local apple varieties growing in Azerbaijan. We were used twenty-nine molecular markers for the scab resistance genes and one molecular marker for the powdery mildew resistance gene. The samples comprised 20 reference scab resistant apple varieties and 5 introduced varieties of apple present in Azerbaijan. Twenty apple-scab resistant varieties and seven introduced apple varieties growing in Azerbaijan were used as objects.

The molecular markers SSR-23.03, Rvi18-SSR, T6, NZmsCN943818 and NH030a of scab resistance genes *Rvi12*, *Rvi18*, *Rvi11*, *Rvi16* and CH03c02 of the powdery mildew resistance gene *PI-d*, were not found in any scab resistant variety we tested and also not in any of the apple varieties originating from Azerbaijan that were tested. The 30 molecular markers we used, proved to be useful for the determination of resistance genes against *V. inaequalis* within apple varieties and can be used plant introgression and pyramidization of resistant genes in the national marker assisted (MAB) breeding programme of Azerbaijan.

Keywords: *Molecular markers, apple varieties, resistance genes, Venturia inaequalis, Podosphaera leucotricha.*

***Corresponding Author:** Elnara Khankishiyeva, Scientific Research Institute of Fruit- and Tea-growing, Ministry of Agriculture, Zardabi settlement, Guba, Azerbaijan, e-mail: elnara_mba@yahoo.de*

Received: *2 September 2020;* ***Accepted:*** *28 November 2020;* ***Published:*** *16 December 2020.*

1. Introduction

Apple scab is a fungal disease caused by *Venturia inaequalis* (Cke.) Wint. This pathogen causes substantial economic losses worldwide (Kaymak, 2012). The damage and losses vary considerably, depending on weather conditions and the sensitivity of varieties used (Jafarov, 2001).

In the spring, especially during the cool and rainy weather, in sensitive varieties disease severity increases, in cases where chemical pesticides are not applied, and crop loss may be up to 100% (Kaymak, 2012). In recent years, due to substantial losses in apple production, in our republic producers have increased pesticide use against apple scab in the cool and humid summers. This, however, increases production costs and it has a negative impact on the environment and human health. Additionally, the pathogen can become resistant to the chemicals used too frequently and excessively http://www.dfnx.gov.az/?r=54&id=218.

To avoid the negative impacts of high pesticide use resistance breeding and the use of resistant varieties has proved to be beneficial (Kaymak, 2012). Resistant genes present in wild apple species have been introduced in commercial varieties using traditional breeding methods. Among the genes that are responsible for resistance to

apple scab, the *Vf* gene is most commonly used, its origin is wild crab apple, *Malus floribunda 821* (Janick *et. al.*, 1996; Williams *et. al.*, 1968). A whole range of resistant genes against *V. inaequalis* were identified in different apple varieties. Vm gene in *Malus micromalus*, Vr gene in *M. pumila*, Vbj gene in *M. baccata kakii*, Vb gene in Hansen's bakkata 2, Va gene in Antonovka PI 172623, Vj gene in Consib and Vc gene in Katay Krab (Williams *et. al.*, 1966). Gygax *et. al.*, (2004), have determined the first molecular markers associated to the applescab resistance gene Vbj, that originated from crab apple *M.baccata* jackii.

Other scab resistance genes are known and for the most part have also been mapped genetically. Bus and Patocchi developed a range of testers consisting of scab-resistant genotypes to identify existing virulence in the field (www.vinquest.ch). Parisi *et. al.*, (1993), reported the appearance of a scab race that is able to overcome Rvi6. This race has now spread across most of Europe, so Rvi6 is no longer effective in many regions of Europe. Despite the breakthrough of this resistant, it can still be important in cultivation (Peil *et. al.*, 2014). The experience that a resistance gene can be overcome by the appearance of a new scab race has led apple growers to try to pyramidize scab resistance genes in one variety (Baumgartner *et. al.*, 2015; Peil *et. al.*, 2014). For most of the scab resistance genes listed in Table 1 there are molecular markers that can be used to select pyramidized resistance in progeny (Patocchi *et. al.*, 2009). However, this requires knowledge of the resistance genes present in the parents. The markers can also be used to screen genetic resources in order to identify resistance donors.

Table 1. Overview of apple scab resistance genes and their localization in the apple genome

Resistance genes		Linkage group	Reference for first report of map position
New nomenclature*	Old name		
Rvi1	Vg	12	Durel *et al.* 2000
Rvi2	Vh2	2	Bus *et al.* 2005
Rvi3	Vh3.1	4	Bus *et al.* 2011
Rvi4	Vh4	2	Bus *et al.* 2005
Rvi5	Vm	17	Patocchi *et al.* 2005
Rvi6	Vf	1	Maliepaard *et al.* 1998
Rvi7	Vfh	8	Bus *et al.* 2011
Rvi8	Vh8	2	Bus *et al.* 2005a
Rvi9	Vdg	2	Bus *et al.* 2011
Rvi10	Va	1	Hemmat *et al.* 2003
Rvi11	Vbj	2	Gygax *et al.* 2004
Rvi12	Vb	12	Erdin *et al.* 2006
Rvi13	Vd	10	Tartarini *et al.* 2004
Rvi14	Vdr1	6	Soufflet-Freslon *et al.* 2008
Rvi15	Vr2	2	Patocchi *et al.* 2004
Rvi16	Vmis	3	Bus *et al.* 2011
Rvi17	Va1	1	Bus *et al.* 2011
Rvi18	V25	1	Soriano *et al.* 2014

Note: * The new nomenclature for scab resistance genes in apple was proposed by Bus et al. 2011.

Generally, in breeding programs it should be tried to produce resistant varieties with market acceptability. In order to successfully develop a breeding program into this direction, apple genotypes which are present in different regions of the country shoud be screened for the presence of resistance. The aim of this study was to identify the

presence of scab resistance in different apple varieties grown in Azerbaijan and to determine valuable varieties for future breeding purposes. For the above-mentioned screening we used twenty seven molecular markers for different scab resistance genes and three molecular marker for the resistance against powdery mildew (*Podosphaera leucotricha*), respectively, that are available from literature (https://sites.unimi.it/camelot/hidras/). During the our study powdery mildew infection was observed in the experimental plot on varieties of Azerbaijan. Therefore we used three molecular markers for powdery mildew resistance.

2. Materials and methods

Plant material sampling. In total 25 different apple varieties were used for molecular screening. In our experiment, 5 introducted varieties of apple were selected from the fruit gardens of the Institute of Fruit-and Tea-growing Research, Guba, Azerbaijan, but 20 donors reference apple varieties from Julius Kühn-Institut (JKI) Dresden, Germany, see Table 2.

Table 2. The apple varieties used in our research

№	The name of variety/ nr of breeding material	Origin
1	Fuji	Japan
2	Kandil Sinap	Turkey or Russia
3	Gala	New Zealand
4	Granny Smith	Australia
5	Aport	Kazakhstan
6	Gala	ACW, Switzerland
7	Golden Delicious	ACW, Switzerland
8	Priscilla	INRA France
9	04214-79, Antonovka APF22	Russia
10	B45	Plant and Food Research, Germany
11	TSR33T239	INRA
12	Durello di Forli	Italy
13	Dülmener Rosenapfel	Germany
14	9-AR2T196	INRA, France
15	TSR34T15	ACW, Switzerland.
16	Hansen`s baccata#2	ACW, Switzerland
17	M.baccata jackii from 2010	ACW, Switzerland
18	A723-6	ACW Switzerland,
19	J34	Plant and Food Research, Germany
20	GMAL2473	ACW Switzerland,
21	Q71	Plant and Food Research, Germany
22	*M.* × *floribunda* 821	Germany
23	06005-55	Germany
24	06006-8	Germany
25	06006-57	Germany

DNA extraction, PCR and fragment analysis. Total DNA was extracted from fresh young leaves (100 mg) using the QIAGEN® DNeasy kit (Qiagen, Mainz, Germany), taking into account the manufacturer's recommendations. To determine the quality and concentration of total DNA obtained, 20 µl DNA samples and a standard range (50 ng, 100 ng, 200 ng, 400 ng) were quantified on 1.0% agarose gel for about 20 minutes at 120 V and subsequently stained with 2 µl ethidium bromide. The evaluation was carried out with the Image ChemiDoc XRS+System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). DNA samples with sufficient purity (A260/A280=1.80-2.00) and concentration (~300–950 ng/µl) were used in PCR.

The DNA was screened with 30 pairs of gene specific primers. The primer sequences for the molecular markers used in our study are given in Table 3.

Table 3. List of primers and sequence characterized used for amplification of scab resistance genes in this study

Marker name	Detected gene	Allele size (bp)	Primer sequence (F+R)	References
Vg15_SSR	*Rvi*1	110	5`-TCGTGCAAGAAGCAA ATAGC-3' 5`-TGGGTTATAATCAAA CCATCCA-3'	Cova *et. al.*, 2015
Vg12_ SSR	*Rvi*1	110	5`-GCTGGGGTTGTTGGA AATAG-3' 5`-TCATCCAAACAAGCA AAACCT-3'	
CH05e03	*Rvi2*, *Rvi4*, *Rvi9*, *Rvi11*	163, 173, 160	5`-CGAATATTTTCACTCT GACTGGG-3' 5`-CAAGTTGTTGTACTGC TCCGAC-3'	Bus *et. al.*, 2005, Gygax *et. al.*, 2004, /Patocchi *et. al.*, 2009
CH02b10	*Rvi2*, *Rvi4*, *Rvi*15	122-125	5`-CAAGGAAATCATCAA AGATTCAAG-3' 5`-CAAGTGGCTTCGGAT AGTTG-3'	Bus *et. al.*, 2005
OPL19 SCAR	*Rvi2* *Rvi8*	430	5`-ACCTGCACTACAATCT TCACT AATC-3' 5`ACTCGTTTCCACTGAGGAT ATTTG-3'	Bus *et. al.*, 2005 / Patocchi *et. al.*, 2009
Hi08e04	*Rvi3*	214?	5`-GCATGGTGGCCTTTC TAAG-3' 5`-GTTTACCCTCTGACTC AACCCAAC-3'	https://sites.unimi.it/camelot/hidras/HiDRAS-SSRdb/pages/marker_display.php?SelectedSSR=Hi08e04
Vr2C5' UTR	*Rvi4* *Rvi15*	521	5`-ATTCATGAGGTCAGC ACCCTC-3' 5`-GCGTAGGCATCAGATA GGACC-3'	Flachowsky, Julius Kühn-Institut (JKI) Dresden, Germany
CH02c02a	*Rvi4* *Rvi15*	176-183	5`-CTTCAAGTTCAGCAT CAAGACAA-3' 5`-TAGGGCACACTTGCT GGTC-3'	Bus *et. al.*, 2005a, /Patocchi *et. al.*, 2009
Hi07h02	*Rvi*5	226	5`-ATTTGGGGTTTCAAC AATGG-3' 5`-GTTTCGGACATCAAA CAAATGTGC-3'	Patocchi *u ðp.*, 2009

FMACH_VM3	*Rvi*5	355	5`-GTTCCCTGCAGTTTCATGGT-3' 5`-CTAGCATTGGCCTCAGATCC-3'	Cova *et. al.*, 2015
FMACH_Vm2	*Rvi*5	158	5`-TGGTGAAAGAAAATATGCCAAG-3' 5`-TCCATTTCTCCATTTGGTGTT-3'	Cova *et. al.*, 2015
CH-Vf1 Rvi17 CH-Vf1c	*Rvi*6 *Rvi*17 *Rvi*19	139-159	5`-ATCACCACCAGCAGCAAAG-3' 5`-CATACAAATCAAAGCACAA CCC-3'	Vinatzer *et. al.*, 2004/ Patocchi *et. al.*, 2009, Bus *et. al.*, 2011
OPB18 SCAR	*Rvi*8	799	5`-CCACAGCAGTCATTGGGA-3' 5`-CCACAGCAGTGCATAAAC-3'	Bus *et. al.*, 2005
CH03d01	*Rvi*9 *Rvi*11	115	5`-CGCACCACAAATCCAACTC-3' 5`-AGAGTCAGAAGCACAGCCTC-3'	Gygax *et. al.*, 2004
T6SCAR	*Rvi*11	410	5`-CGTTCAACTCATAAGTGGT CCC-3' 5`-AAGGGCAGAATCATAAAAGCC-3'	Gygax *et. al.*, 2004
SSR23.03	*Rvi*12	106	5`-CAGTGCTGGCTTTAAGTTTGG -3' 5`-AATACAACGCCAGATGAGAG G-3'	Padmarasu *et. al.*, 2014
SSR-24.91	*Rvi*12	209	5`-CTTGCTAGGGTTGTGCTTGG-3' 5`-CCACATAAAAGAAAGCCTTGG-3'	Padmarasu *et. al.*, 2014
SSR-23.17	*Rvi*12	242	5`-GTTGCCCGTTAGAATTTTGC-3' 5`-CTAGTGTAGTGTGTGGGTGTGG-3'	
CH02c06	*Rvi*12	248	5`-TGACGAAATCCACTACTAATGCA-3' 5`-GATTGCGCGCTTTTTAACAT-3'	Gianfrance-schi *et. al.*, 1998
CH02b07	*Rvi*13	120	5`-CCAGACAAGTCATCACAACACTC-3' 5`-ATGTCGATGTCGCTCTGTTG-3'	Tartarini *et. al.*, 2004/ Patocchi *et. al.*, 2009
CH04f03	*Rvi*13	191	5`-CTTGCCCTAGCTTCAAATGC-3' 5`-TCGATCCGGTTAGGTTTCTG-3'	Tartarini *et. al.*, 2004 / Patocchi *et. al.*, 2009
HB09	*Rvi*14	210	5`GCTCAAAATACTGAAGCCTTGC-3' 5`-GGGGAAGCAGGATGGTTACT-3'	Soufflet-Freslon *et. al.*,2008,/Patocchi *et. al.*, 2009
CH02f06	*Rvi*15	152	5`-CCCTCTTCAGACCTGCATATG-3' 5`-ACTGTTTCCAAGCGCTCAGG-3'	Patocchi *et. al.*, 2004/ Patocchi *et. al.*, 2009

NZmsCN 943818	*Rvi*16	198	5`-CGGGAAGAGGAAAT GTGATT-3' 5`-TGAACAGCTCATCGT CGGTA-3'	Bus *et. al*., 2010, Celton *et. al*., 2009
NH030a	*Rvi*16	210	5`-GCAACAGATAGGAG CAAAGAGGC-3' 5`-TCCAAAGTTCAACAC AGATCAAGAG-3'	Bus *et. al*., 2010, Yamamoto *et. al*., 2002 Celton *et. al*., 2009
Rvi18-SSR	*Rvi*18	478	5`-GGTTTTCATTCTTGCA TGAGG -3' 5`-GTTTTCGACGAACTC CTAACT TCACC-3'	Soriano *et. al*., 2014
AT20-450 SCAR	*Pl*1	450	5`-ATCAGCCCCACATGA ATCTCATACC-3' 5`-ACATCAGCCCTCAAA GATGAGAAGT-3'	Markussen *et. al*., 1995, Frey *et. al*., 2004
CH02d12	*Pl*m	205	5`-AACCAGATTTGCTTG CCATC-3' 5`-GCTGGTGGTAAACGT GGTG-3'	Gardiner *et. al*., 2003
CH03c02	*Pl*d	133	5`-TCACTATTTACGGGA TCAAGCA -3' 5`-GTGCAGAGTCTTTGA CAAGGC-3'	James, *et. al*., 2004 Seglias, *et. al*., 1997
Pl2_F/R	*Pl*2	252	5′-CTGCTCTTCCACATG TACCT-3′ 5′-TAAGAGCACTGTTCT TAGTGG-3′	

Six multiplex mixed fluorescent markers (100 mM) were prepared to be run on an ABI 3500 X L (genetic analyzer DNA Hitachi, Tokyo, Japan)). Protocols for each multiplex PCR (MP) were obtained using the Type-it Microsatellite PCR kit (Qiagen, Germany). For each sample, a solution consisting of 1xMM (Master Mix), 1 mM Q-Solution (Q), 1 mM multiplex mixture, 1µl ddH2O and 2µl of DNA was prepared. The following PCR conditions were used: initial denaturation at 95°C for 5 min, then 40 cycles at 95°C for 30 s., 58°C for 1 min 30 s. and 72°C for 1 min., followed by 60°C for 30 min and a final extension of 60° for 30 min. Amplifications were performed in a gradient PCR Thermal Cycler (FlexCycler, Analytic Jena). Fragment analysis was performed on the ABI 3500XL capillary sequencer (Applied Biosystems) according to the manufacturer's instructions, 1 µl of PCR reaction was diluted with 8,95 µl HiDi formamide, 0,05 µl 600-LİZ (Applied Biosystems) and denatured for 5 min at 95°C. After sequencing, genotype analysis (scorable peak assignment) was performed using the software package GeneMapper® 5.0 and analyzed using the default values.

3. Results and discussion

In our experiments, we determined the molecular markers of resistance genes to apple scab within 25 varieties of apple. But during the our study powdery mildew lesion was observated in varieties of Azerbaijan. Therefore we used one molecular markers for the powdery mildew resistance . The marker Hi08e04 was used for identification of gene Rvi3 in 25 accessions of apple varieties. This marker also was found in scab resistant cultivar Q71 (Geneva × Braeburn). Next, the molecular markers of scab resistance gene Rvi1 was detected in 1 scab resistant cultivar. These molecular markers

Vg12_SSR and Vg15_SSR have been developed for Rvi1 by Cova *et. al.*, (2015). These markers are mapped on apple linkage group 12 and being placed at 0.12 cM from Rvi1. This scab resistance genes were identified in 5 Azerbaijan apple varieties: Gala, Granny Smith, Kandil Sinap, Aport, Fuji. But marker Vg15_SSR of Rvi1 scab resistance gene was detected introducted cultivar Gala. The The SSR molecular markers CH05e03 and CH03d01 have been developed for Rvi11 by Gygax *et. al*., 2004. These markers are mapped on apple linkage group 2 (Patocchi *et. al.*, 2005). The marker CH05e03 (SSR) being placed at 0.6 cM from Rvi11. This marker of Rvi2, Rvi4, Rvi9 and Rvi11 scab resistance genes were detected in 4 apple varieties TSR34T15, TSR33T239, J34, *M.baccata* jackii, but molecular marker CH03d01 Rvi9 and Rvi11 scab resistance genes were detected in 2 apple varieties (Table 4). The gene Rvi12 was revealed by CH02c06 marker in *Hansen`s baccata* accession including. The marker OPB18SCAR have been developed for Rvi8 by Bus *et. al.*, (2005a). This SCAR marker was detected in B45 scab resistant cultivar. The marker CH04f03 has been developed for Rvi13 by Tartarini *et. al.*, 2004. This marker is mapped on apple linkage group 10 (Patocchi *et. al.*, 2005). The SSR marker of Rvi13 scab resistance gene were detected in 2 varieties Durello di Forli and Apor (Table 4). The marker CH02b10 have been developed for Rvi4 by Bus *et. al.*, 2005. This gene was mapped on linkage group 2. This SSR marker was detected in TSR34T15 scab resistant cultivar. Next, the molecular markers of scab resistance gene Rvi13 was detected in 5 scab resistant cultivar. This marker is mapped on apple linkage group 10 (Patocchi *et. al.*, 2005). But our results were different to published data for previously studied Durello di Forli cultivar (Patocchi *et. al.*, 2005; (Tartarini *et. al.*, 2004). Marker CH02b07 amplified two fragments 111 bp and 126 bp was observed in scab resistant cultivar Durello di Forli. Marker CH-Vf1 of Ri6 scab resistance gene was detected only in Priscilla, *Malus x floribunda* 821, Antonovka APF22 varieties (Table 4). These molecular markers have been developed by Vinatzer *et. al.*, (2004). Rvi6 and Rvi17 scab resistance genes have been closely mapped on LG1 according to the information given by Gessler *et. al.*, (2006). Patzak *et. al.*, (2011), did not detect the molecular marker of the Rvi17 resistance gene in scab resistant cultivar Antonovka. According to their information the cross-reaction between the molecular markers of Rvi6 resistance gene and Rvi17 resistance gene was not confirmed. The molecular markers FMACH_VM2 and FMACH_VM3 have been developed for Rvi5 by Cova *et. al.*, (2015). The SSR marker of Rvi5 scab resistance gene was detected in 1 apple genotype 9-AR2T196. The molecular markers SSR-23.17 and SSR-24.91 have been developed for Rvi12 by Padmarasu *et. al.*, (2014). The markers of Rvi12 scab resistance gene was detected in 1 scab resistance genotype Hansen`s baccata. The molecular markers CH02f06, Vr2C5'UTR and CH02c02a have been developed for Rvi15 by Patocchi *et. al.*, (2004). The SSR markers CH02f06 of Rvi15 scab resistance gene was detected in 10 apple varieties (TSR34T15, TSR33T239, Priscilla, J34, GMAL2473, Gala, Granny Smith, Kandil Sinap, Aport and Fuji) and CH02c02a Rvi4 scab resistance gene was identified in 2 apple varieties (TSR33T239, GMAL2473). The gene Rvi4 (Rvi15) was revealed by Vr2C5'UTR marker in 2 scab resistant varieties GMAL2473 and TSR33T239. The molecular marker Hi07h02 has been developed for Rvi5 by Patocchi *et. al.* 2009. This marker Hi07h02 of Rvi5 scab resistance gene was detected in 1 scab resistant cultivar 9-AR2T196. The gene Rvi14 was revealed by Vr2C5'UTR marker in 1 scab resistant cultivar Dülmener Rosenapfel. The AT20Scar molecular marker of the powdery mildew resistance gene PI1 was detected in 06005-55, 06006-8, 06006-57, Hansen`s baccata and J34 (Table 4).

Table 4. Detection of molecular markers (SSR and SCAR) of resistance genes within varieties of apple

Name of cultivar	Scab resistance marker																						
	CH02c02a	Hi07h02	Pl2_F/R	HB09	OPL19SCAR	FMACH_VM3	Vr2C5UTR	CH03d01	SSR-24.91	CH02f06	SSR-23.17	FMACH_Vm2	CH-Vf1	CH02b07	AT20Scar	CH02b10	CH04f03	OPB18SCAR	CH02c06	CH05e03	CH02d12	Vg15_SSR	Vg12_SSR	Hi08e04
Fuji					+					+													+	
Kandil Sinap					+					+													+	
Gala					+					+												+	+	
Granny Smith					+					+													+	
Aport					+					+							+						+	
Royal Gala			+																					
Golden Delicious																						+	+	
TSR34T15			+		+					+						+				+				
Q71 (Geneva x Braeburn)			+																					+
TSR33T239	+		+				+			+										+				
9-AR2T196		+	+			+						+												
Priscilla			+							+			+	+										
M. x floribunda 821			+										+											
B45			+		+													+						
J34			+					+		+				+	+					+				
A723-6			+																					
Hansen`s baccata#2			+						+		+				+				+					
M.baccata jackii from 2010			+					+												+				
Durello di Forli			+											+			+							
Dülmener Rosenapfel			+	+										+										
GMAL2473	+		+				+			+														
04214-79, Antonovka APF22			+										+	+										
06005-55			+												+									
06006-8			+												+						+			
06006-57			+												+						+			

The AT20Scar molecular marker of the powdery mildew resistance gene PI1 was detected in 5 apple genotypes (J34, Hansen`s baccata#2, 06005-55, 06006-8, 06006-57), Pl2_F/R molecular marker of the powdery mildew resistance gene PI2 was detected in 19 apple genotypes, but CH02d12 molecular marker of the powdery mildew resistance gene Plm was identified in apple genotypes 06006-8 and 06006-57. The SCAR molecular marker OPL19 have been developed by Bus *et al.* 2005b. Rvi2 and Rvi8 scab resistance genes have been closely mapped on LG2 (Baumgartner *et. al.*, 2015). This marker OPL19 of Rvi2 and Rvi8 scab resistance genes were detected in scab resistant varieties TSR34T15 and B45. This scab resistance genes were identified in 5 Azerbaijan apple varieties.

The molecular markers SSR-23.03, Rvi18-SSR, T6, NZmsCN943818 and NH030a of scab resistance genes Rvi12, Rvi18, Rvi11, Rvi16; CH03c02 powdery mildew resistance gene PI-d were not found in any scab resistant varieties, which we used them in our experiment. Some markers like of these resistance genes were not able to distinction between resistant and susceptible varieties, the main reason for that, the sources of this genes have not been incorporated into new apple varieties (Erdin *et. al.*, 2006; MacHardy, 1996). Also were absent in any apple varieties of the Azerbaijan, we were not able to detect these markers and as well molecular marker CH-Vf1 of the Rvi6 scab resistance gene. We think that, in our studied resistant varieties can be exploited easily to breed scab resistance varieties using the aid of marker assisted backcrossing.

Furthermore, the assessment of apple scab lesion in the greenhouse was made to Chevalier *et al.* (1991) was evaluated based on a 4-point scale and was with fragment analysis results a comparative. Of Azerbaijan apple varieties demonstrated 3-resistant, 1-moderate susceptibility and 1-high susceptibility.

4. Conclusion

Our country has an important place in terms of apple genetic resources. The results obtained show that, the Azerbaijan introduction varieties a variability of gene specific markers to against pathogen. Considering the current values, the development of quality apple varieties which are suitable for production areas, maturing at different periods and resistant to common diseases such as scab, will provide high added value to the country's economy and benefit to environment and human health. In the future new sources of resistance can be used in resistance breeding programs.

References

Baumgartner, I., Kellerhals, M., Costa, F. et. al. (2016). Development of SNP-based assays for disease resistance and fruit quality traits in apple (Malus × domestica Borkh.) and validation in breeding pilot studies. *Tree Genetics & Genomes, 12*(3), 12-35. doi:10.1007/s11295- 016-0994-y.

Baumgartner, I.O., Patocchi, A., Frey, J.E., Peil, A., Kellerhals, M. (2015). Breeding elite lines of apple carrying pyramided homozygous resistance genes against apple scab and resistance against powdery mildew and fire blight. *Plant Molecular Biology Reporter, 33*(5), 1573-1583. doi:10.1007/s11105-015-0858-x.

Boudichevskaja, A., Flachowsky, H., Peil, A., Fischer, C., Dunemann, F. (2006). Development of a multiallelic SCAR marker for the scab resistance gene Vr1/Vh4/Vx from R12740-7A apple and its utility for molecular breeding. *Tree Genetics & Genomes,* 2, 186-195. doi: 10.1007/s11295-006-0043-3.

Bus, V.G.M., Bassett, H.C.M., Bowatte, D. et. al. (2010). Genome mapping of an apple scab, a powdery mildew and a woolly apple aphid resistance gene from open-pollinated Mildew Immune Selection. *Tree Genetics & Genomes,* 6, 477-487. doi:10.1007/s11295-009-0265-2.

Bus, V.G.M., Laurens, F.N.D., van de Weg, W.E., Rusholme, R.L., Rikkerink, E.H.A., Gardiner, S.E. et. al. (2005). 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 Phytologist, 166*(3), 1035-1049. doi: 10.1111/j.1469-8137.2005.01395.x.

Bus, V.G.M., Rikkerink, E.H.A., Caffier, V., Durel, C.E., Plummers, K.M. (2011). Revision of nomenclature of the differential host-pathogen interactions of Venturia inaequalis and

Malus. *Annual Review of Phytopathology*, 49, 391-413. doi: 10.1146/annurev-phyto-072910-095339.

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. (2005). 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. doi: 10.1007/s11032-004-3609-5.

Celton, J.M., Chagne, D., Tustin, D.S., Gardiner, S.E. (2009). Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from Malus ESTs and Pyrus genomic sequences. *In Tree Genetics & Genomes, 5*(1), 93-107. doi: 10.1007/s11295-008-0171-z.

Chevalier, M., Lespinasse, Y., Renaudin, S. (1991). A microscopic study of different classes of symptoms coded by the Vf gene in apple for resistance to scab (Venturia inaequalis). *Plant Pathology,* 40, 249-256. doi.org/10.1111/j.1365-3059.1991.tb02374.x.

Cova, V., Lasserre-Zuber, P., Piazza, S., Cestaro, A., Velasco, R., Eric, D.Ch., Malnoy, M. (2015). High-resolution genetic and physical map of the Rvi1 (Vg) apple scab resistance locus. *Mol Breeding, 35*(16), 1-13. doi: 10.1007/s11032-015-0245-1.

Dunemann, F., Peil, A., Urbanietz, A., Garcia-Libreros, T. (2007). Mapping of the apple powdery mildew resistance gene Pl1 and its genetic association with an NBS-LRR candidate resistance gene. *Plant Breeding*, 126, 476-481. doi.org/10.1111/j.1439-0523.2007.01415.x.

Durel, C., Van de Weg, E., Venisse, J., Parisi, L. (2000). Localisation of a major gene for apple scab resistance on the European genetic map of the Prima x Fiesta cross. *IOBC WPRS Bulletin, 23*(12), 245-248.

Erdin, N., Tartarini, S., Broggini, G., Gennari, F., Sansavini, S., Gessler, C. et al. (2006). Mapping of the apple scab-resistance gene Vb. *Genome, 49*(10), 1238-1245. doi.org/10.1139/g06-096

Frey, J.E., Frey, B., Sauer, C., Kellerhals, M. (2004). Efficient low-cost DNA extraction and multiplex fluorescent PCR method for marker-assisted selection in breeding. *Plant Breeding*, 123, 554-557. doi.org/10.1111/j.1439-0523.2004.01033.x.

Gardiner, S.E., Murdoch, J., Meech, S., Bus, V., Rusholme, R., Rikkerink, E., Bassett, H., Cook, M., Gleave, A., Crowhurst, R., Ross, G., Warrington, I. (2003). Candidate resistance genes from an EST database prove a rich source of markers for major genes conferring resistance to important apple pests and diseases. *Acta Horticulturae,* 622, 141 - 151.

Gessler, C., Patocchi, A., Sansavini, S., Tartarini, S., Gianfranceschi, L. (2006). Venturia inaequalis resistance in apple. *Critical Reviews in Plant Science*, 25, 473–503.

Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M., Gessler, C. (1998). Simple sequence repeats for the genetic analysis of apple. *Theor Appl Genet*, 96, 1069-1076. doi.org/10.1023/A:1015677505602

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.

Hemmat, M., Brown, S., Aldwinckle, H., Weeden, N., Mehlenbacher, S. (2003). Identification and mapping of markers for resistance to apple scab from 'Antonovka' and 'Hansen's baccata# 2'. *Acta Horticulturae*, 622, 153-161. doi:10.17660/ActaHortic. 2003.622.13.

Jafarov, I. (2001). Agricultural Phytopathology. Baku, *Science publishing house*, 277.

James, C.M., Evans, K.M. (2004). Identification of molecular markers linked to the mildew resistance genes Pl-d and Pl-w in apple. *Acta Horticulturae*, 663, 123 – 127. doi.org/10.1007/s00122-004-1836-0.

Janick, J., Moore, J.N. (eds.). (1996). Fruit breeding. Vol I. Tree and Tropical Fruits. John Wiley & Sons, Inc., New York, USA, 77.

Jones, A.L., Aldwinckle, H.S. (1991). Compendium of apple and pear diseases. *The American Phytopathological society 3340 Pilot Knob Poad st. Paul, Minnesota 55121-2097, USA. 53*, 224.

Kaymak, S. (2012). Apple scab disease caused by Venturia inaequalis [(Cooke) G. Winter1875] Turkey isolates determination of molecular characterization and pathogenicity. Ph.D Thesis, Turkey, 5-13.

MacHardy, W.E. (1996). Apple Scab - Biology, Epidemiology, and Management St.Paul, Minnesota: *The American Phytopathological Society*, 545.

Maliepaard, C., Alston, F., Van Arkel, G., Brown, L., Chevreau, E., Dunemann, F. et al. (1998). Aligning male and female linkage maps of apple (Malus pumila Mill.) using multi-allelic markers. *TAG Theoretical and Applied Genetics, 97*(1), 60-73.

Markussen, T., Kruger, J., Schmidt, H., Dunemann, F. (1995). Identification of PCR-based markers linked to the powdery-mildew resistance gene Pl1 from Malus robusta in cultivated apple. *Plant Breeding, 114*, 530-534. doi.org/10.1111/j.1439-0523.1995.tb00850.x.

Padmarasu, S., Sargent, D.J., Jaensch, M., Kellerhals, M., Tartarini, S., Velasco, R., Troggio, M., Patocchi, A. (2014). Fine-mapping of the apple scab resistance locus Rvi12 (Vb) derived from 'Hansen's baccata# 2'. *Mol Breeding*, 34, 2119-2129. doi: 10.1007/s11032-014-0167-3.

Parisi, L., Lespinasse, Y., Guillaumes, J., Kruger, J. (1993). A new race of Venturia inaequalis virulent to apples with resistance due to the Vf gene. *Phytopathology, 83*(5), 533-537. doi: 10.1094/Phyto-83-533.

Patocchi, A., Bigler, B., Koller, B., Kellerhals, M., Gessler, C. (2004). Vr(2): a new apple scab resistance gene. *Theoretical and Applied Genetics, 109*(5), 1087-1092. doi:10.1007/s00122-004-1723-8.

Patocchi, A., Fernández-Fernández, F., Evans, K., Gobbin, D., Rezzonico, F., Boudichevskaia, A., Dunemann, F., Stankiewicz-Kosyl, M., Mathis-Jeanneteau, F., Durel, C.E., Gianfranceschi, L., Costa, F., Toller, C., Cova, V., Mott, D., Komjanc, M., Barbaro, E., Kodde, L., Rikkerink, E., Gessler, C., van de Weg, W.E. (2009). Development and test of 21 multiplex PCRs composed of SSRs spanning most of the apple genome. *Tree Genetics & Genomes, 5*(1), 211 – 223. doi:10.1007/s11295-008-0176-7.

Patocchi, A., Frei, A., Frey, J.E., Kellerhals, M. (2009). Towards improvement of marker assisted selection of apple scab resistant varieties: Venturia inaequalis virulence surveys and standardization of molecular marker alleles associated with resistance genes. *Molecular Breeding, 24(4),* 337-347. doi:10.1007/s11032-009-9295-6.

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. doi:10.1139/g05-036.

Patzak, J., Paprštein, F., Henychová, A. (2011). Identification of Apple Scab and Powdery Mildew Resistance Genes in Czech Apple (Malus × domestica) Genetic Resources by PCR Molecular Markers. *Genet. Plant Breed, 47*(4), 156–165.

Peil, A., Dunemann, F., Richter, K., Höfer, M. et al. (2008). Resistance breeding in apple at Dresden-Pillnitz. In: Weinsberg FÖOEV, ed. *Proceedings of the 13th International Conference on Cultivation Technique and Phytopathological Problems in Organic Fruit-Growing, Weinsberg. 220*(5), 220-225.

Peil A, Kellerhals M, Rueß F, Baab G, Mayr U. (2014). Schorfresistente Sorten: Nach wie vor ein wichtiger Baustein zur nachhaltigen Obstproduktion. *Obstbau, 39*, 131.

Seglias, N., Gessler, C. (1997). Genetics of apple powdery mildew resistance from Malus zumi (Pl2). IOBC (WPRS) Bulletin: *Integrated Control of Pome Fruit Diseases, 20*, 195 -208.

Soriano, J.M., Madduri, M., Schaart, J.G., van der Burgh, A., van Kaauwen, M.P.W., Tomic, L., Groenwold, R., Velasco, R., van de Weg, E., Schouten, H.J. (2014). Fine mapping of the gene Rvi18 (V25) for broad-spectrum resistance to apple scab, and development of a

linked SSR marker suitable for marker-assisted breeding. *Molecular breeding, 34*(4), 2021-2032. doi:10.1007/s11032-014-0159-3 handle.

Soufflet-Freslon, V., Gianfranceschi, L., Patocchi, A., Durel, C.E. (2008). Inheritance studies of apple scab resistance and identification of Rvi14, a new major gene that acts together with other broad-spectrum QTL. *Genome, 51*(8), 657-667. doi:10.1139/G08-046.

Tartarini, S., Gennari, F., Pratesi, D., Palazzetti, C., Sansavini, S., Parisi, L. et. al. (2004). Characterisation and genetic mapping of a major scab resistance gene from the old Italian apple cultivar 'Durello di Forli'. *Acta Horticulturae, 663,* 129-134. doi:10.17660/ActaHortic.2004; 663.16.

Urbanovich, O., Kazlovskaya, Z. (2008). Identification of scab resistance genes in apple trees by molecular markers. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. *Sodininkyste ir Daržininkyste,* 27, 347–357.

Vinatzer, B.A., Patocchi, A., Tartarini, S., Gianfranceschi, L., Sansavini, S., Gessler, C. (2004). Isolation of two microsatellite markers from BAC clones of the Vf scab resistance region and molecular characterization of scab resistant accessions in Malus germplasm. *Plant Breed. (in press), 123*(4), 321–326.

Williams, E.B., Brown, A.G. (1968). A new physiologic race of Venturia inaequalis, incitant of apple scab. *Plant Disease reporter, 52*(10), 799-801.

Williams, E.B., Kuc, J. (1966). Resistance in Malus to Venturia inaequalis. *Annual Review of Phytopathology, 7,* 223-246.

Yamamoto, T., Kimura, T., Shoda, M., Imai, T., Saito, T., Sawamura, Y., Kotobuki, K., Hayashi, T., Matsuta, N. (2002). Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theor. Appl. Genet., 106*, 9-18.