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For the determination of resistance against pathogenic *Venturia inaequalis* (CAKE.), using molecular markers, in selected of apple in Azerbaijan

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Abstract
To evaluate selected varieties of apple, in Azerbaijan resistant against *Venturia inaequalis* (CKE.) Wint, for the determination of the presence of resistance markers, using molecular method. We used 30 markers for the scab resistance genes. For the samples have been used 8 selected varieties of apple of Azerbaijan and 20 scab resistant apple. Results indicate that, the OPL19SCAR marker for the major resistance genes Rvi2 and Rvi8 were detected 7 apple varieties, marker Vg12_SSR of the Rvi1 scab gene in all apple varieties, Vg15_SSR marker only 1 varieites, marker CH02f06 of the Rvi15 resistance gene in all varieties, marker CH02c02a of the Rvi4 and Rvi15 scab resistance genes in 1 varieties, marker CH05e03 of the Rvi2, Rvi4, Rvi9 and Rvi11 scab genes 1 varieties, marker CH02b07 of the Rvi13 resistance gene only in 2 varieties, marker CH03d01 of the Rvi9 and Rvi11 scab genes in 1 varieties and CH04f03 of the Rvi13 resistance genes in 2 were detected selected varieties from Azerbaijan. The importance of SSR and SCAR molecular markers, which we used in our research for the detection in the apple varieties of resistance genes against *Venturia inaequalis* was confirmed. From these SSR and SCAR molecular markers can be used in plant pyramidation of scab resistance genes in our republic's National Marker Assistant (MAB) selection program.

Keywords: resistance genes, SSR and SCAR markers, apple varieties, *Venturia inaequalis*

İntroduction
İn our country (especially in Guba, Khachmaz and Gusar region), the pathogen *Venturia inaequalis* is a fungal disease that causes changes in the size and shape of fruits, premature shedding of young leaves (Kaymak 2012) [24]. The losses and damage is changing, the sensitivity of varieties used and depending on the weather conditions (Jafarow 2001) [20].
According to scentific studies conducted by various researchers around the world, the Rvi6 gene is the most resistance gene against the pathogen *Venturia inaequalis*. According by Janick et al. (1996) [22], Williams et al. (1968) [44], the information they gave in different years, the origin of this gene is red to be a wild crab apple.
As a result of molecular studies conducted around the world, the Rvi1 (Vg) gene Golden Delishes (Durel *et al.* 2000) [12], Rvi2 (Vh2) TSR34T15 (Bus *et al.* 2005) [7], Rvi3 (Vh3.1) Geneva (Bus *et al.* 2011) [6], Rvi4 (Vh4) gene TSR33T239 (Bus *et al.* 2005) [7], Rvi5 (Vm) gene 9-AR2T196 (Patocchi *et al.* 2005) [34], Rvi6/Rvi7 (Vf / Vfh) gene Malus floribunda 821 (Maliepaard *et al.* 1998; Bus *et al.* 2011) [27, 6], Rvi8 (Vh8) gene B45 (Malus sylvestris W193b) (Bus *et al.* 2005a) [5], Rvi9 (Vdg) gene K2 (Bus *et al.* 2011) [6], Rvi10 (Va) gene Antonovka (A723-6) (Hemmat *et al.* 2003) [19], Rvi11 (Vbj) gene Malus bakkata jackii (Gygax *et al.* 2004) [18], Rvi12 (Vbj) gene Hansen's bakkata (Erdin *et al.* 2006) [13], Rvi13 (Vdg) gene Durello di Forli (Tartarini *et al.* 2004) [41], Rvi14 (Vdr1) Dülmener Rosenapfel (Soufflet-Freslon *et al.* 2008) [40], Rvi15 (Vr2) gene GMAL2473 (Patocchi *et al.* 2004) [31], Rvi16 (Vmis) MIS op 93.051 G07-098 (Bus *et al.* 2011) [6] and Rvi17 (Va1) Antonovka APF22 (Bus *et al.* 2011) [6] were found in genotypes.
Thus, based on the analysis of the literature, it should be noted that the conduct of molecular research to determine the genes of resistance of apple varieties to scab in our country has been neglected. For this purpose, in 2018, for the first time, we began to conduct research to identify the genes of resistance with molecular markers SSR and SCAR to apple scab diseases of apple varieties grown in our country.

Materials and methods
Plant sampling. We were taken as the material of our research, eight selection apple varieties the Fruit and Tea Growing Research Institute of Azerbaijan and twenty donor apple varieties available at the Julius Kühn-Institute (JKI) of Germany. We also used these 20 apple varieties as donor material in our study on the detection of genes for resistance against scab to apple varieties introduced in our country (Khankishiyeva 2020) [25].

Table 1: A short description of the varieties

Varieties	The origin of varieties	The parentage of varieties
Azerbaijan	Azerbaijan	Landsberg renette x Payizlig Kludius
Naile	Azerbaijan	Sari tursh x Golden Delicious
Elwin	Azerbaijan	Guba renette x Sari tursh
Dawamli	Azerbaijan	Naile x Cir Haci x Shampan renette
Payizlig Guba	Azerbaijan	London Pepping x 3-7-21
Sulh	Azerbaijan	Shampan renette x Gishlig gizil Parmen
Gobustan	Azerbaijan	Naile x Cir Haci x Sari tursh
Gizil tac	Azerbaijan	Semed Vurgun x Cir Haci
Gala*	Switserland.	
Golden delicious*	Switserland.	
Priscilla*	France (Inra)	
Antonovka*	Russia	
B45*	Germany	Pacific beauty x Malus sieversii, GMAL 4302.x 8
TSR33T239*	Russia	
Durello di forli*	Italy.	
Dülmener rosenapfel*	Germany	
9AR2T.196*	France (Inra)	
TSR34T.15*	Switserland.	
*Hansen`s baccata**	Switserland.	
*Malus baccata**	Switserland.	
A723.6*	Switserland.	Worcester pearmain x Pl.172623
J34*	Germany	Gala x Dolgo
GMAL2473*	Switserland.	
Q71*	Germany	Geneva x Braeburn
Malus floribunda 821.*	Germany	
06005.55*	Germany	
06006.8*	Germany	
06006.57*	Germany	

*This donor varieties have also been used in our other research (Khankishiyeva 2020) [25]

DNA extraction, PCR and fragment analysis methods. First of all, we were extracted the DNA of apple varieties used in our research (QIAGEN® DNeasy kit, Qiagen, Germany).

To determine the concentration and quality of DNA samples obtained during the study, they were measures in 1.0 % Agariose gel (2 µl Etidium bromide). The result were evaluated then with Image ChemiDoc XRS (Hercules, CA, USA). In our study, we used 25 SSR and 5 SCAR molecular markers to identify resistance genes against scab. We also used SSR and SCAR markers in our study to identify genes for resistance against scab in introduced to apple varieties introduced to our country (Khankishiyeva 2020) [25]

On the fragment analysis, a solution of 1 mM Q-solution, 1 mM multiplex mixture, 1xMM, 1 µl ddH_20, 2 µl DNA was prepared for each samples (ABI 3500 X L – Hitachi, Japan). Then denature for 5 min. 95^0C, then 40 cycles for 30 seconds at 95^0C, 1 minute 30 seconds at 58^0C, for 1 minute 72^0C, followed for 30 minutes 60^0C, final extension for 30 minutes 60^0C. In our study, we used in 0.05 µl 600 LIZ and 8.95 µl HiDi formamide for dilution in fragment analysis. After that, we carried out the denaturation process for 5 minutes at a temperature of 95^0 C. The final results were evaluated using the software GeneMapper 5.0 (Khankishiyeva 2020) [25].

The following table provides information on the characteristics of the molecular markers used in our study and the size of alleles identified by various researchers. We have used these markers in the detection of resistance genes against the pathogen *Venturia inaequalis* of varieties cultivated in large areas, introduced to our country in recent years. It should be noted, given that in our study, SSR and SCAR markers have all the superior features, we used them to identify resistance genes of apple genotypes to scab. However, SSR markers differ from SCAR markers in that they are technically faster, easier, and co-dominant.

Table 2: Characterized of primers for amplification of apple scab resistance genes in research (Khankishiyeva 2020) [25].

№	Marker name	Resistance gene	Size of allele (bp)	References
1	Vg_12SSR	Rvi1	110	Cova *et al.* (20 15)[10]
2	Vg_15SSR	Rvi1	110	
3	CH05e03	Rvi2, Rvi4, Rvi9, Rvi11	163, 160, 173	Bus *et al.* (2005)[7] Gygax *et al.* (2004)[18] Patocci *et al.* (2009)[33]

4	CH02b10	Rvi2, Rvi4, Rvi15	122, 125	Bus *et al.* (2005)[5]
5	OPL19SCAR	Rvi2, Rvi8	4 3 0	Bus *et al.* (2005)[5] Patocci *et al.* (2009)[33]
6	Hi08 e 04	Rvi3	2.1 4	https://sites.unimi.it/camelot/ hidras
7	Vr2C5'Utr	Rvi4, Rvi15	5 2 1	Flachowsky, Julius Kühn-Institut (JKI) Dresden, Germany
8	CH02c02a	Rvi4, Rvi15	176, 183	Bus *et al.* (2005)[7] Patocci *et al.* (2009)[33]
9	Hi07h02	Rvi5	226	Patocci *et al.* (2009)[33]
10	FMACH_vm 3	Rvi5	355	Cova *et al.* (20 15)[10]
11	FMACH_vm 2	Rvi5	158	Cova *et al.* (20 15)[10]
12	CH-Vf1	Rvi6, Rvi17, Rvi19	139, 159	Vinatzer et *al.* (2004) [43] Patocci *et al.* (2009)[33] Bus *et al.* (2011)[6]
13	OPB18SCAR	Rvi8	799	Bus *et al.* (2005)[5]
14	CH03d01	Rvi9, Rvi11	115	Gygax *et al.* (2004)[18]
15	T6SCAR	Rvi11	410	Gygax *et al.* (2004)[18]
16	SSR23.03	Rvi12	106	Padmarasu et al. (2014)[29]
17	SSR24.91	Rvi12	209	Padmarasu et al. (2014)[29]
18	SSR23.17	Rvi12	242	
19	CH02c06	Rvi12	248	Gianfranceschi *et al.* (1998) [17]
20	CH02b07	Rvi13	120	Tartarini et al. (2004) [41] Patocci *et al.* (2009) [33]
21	CH04f03	Rvi13	191	Tartarini et al. (2004) [41]Patocci *et al.* (2009) [33]
22	HB09	Rvi14	210	Soufflet *et al.* (2008) [40] Patocchi *et al.* (2009) [33]
23	CH02f06	Rvi15	152	Patocci *et al.* (2004) [31] Patocci *et al.* (2009) [33]
24	NZmsCN943 818	Rvi16	198	Bus *et al.* (2010) [4] Celton *et al.* (2009) [8]
25	NH030a	Rvi16	210	Bus *et al.* (2010)[4] Celton *et al.* (2009) [8] Yamamato *et al.* (2002) [46]
26	RVI18SSR	Rvi18	478	Soriano *et al.* (2014) [39]
27	AT20.450 SCAR	Pl1	450	Markussen *et al.* (1995)[28] FREY *et al.* (2004)[14]
28	CH02d12	Plm	205	Gardiner *et al.* (2003) [15]
29	CH03c02	Pld	133	James *et al.*(2004) [22] Seglias *et al.* (1997) [38]
30	PL12_F/R	Pl2	252	

Results and Discussion

As a result of fragment analysis, we found markers of resistance genes against scab in eight selection and twenty donor apple varieties used as research material. Since we have provided detailed information on genes and markers above, we will only provide information on which markers have been identified for the scab resistance genes in the varieties we have used in our study. The marker Hi08e04 of the major scab resistance gene Rvi3 and marker Vg15_SSR of the gene Rvi1 have been not found in any of the selected varieties in our study. However, in another study we conducted, this result was different. Thus, the marker Vg15_SSR of gene Rvi1 has been in the introduced Gala variety (Khankishiyeva 2020) [25]. The marker Vg12_SSR of the gene Rvi1 has been found Dawamli, Sulh, Gizil tac, Azerbaijan, Gobustan, Elwin, Payizlig Guba, but found not in Naile selected variety. However, this marker has been found in all introduced varieties used in our other study (Khankishiyeva 2020) [25]. The marker CH05e03 of the gene Rvi11 has been found in selected varietz Gizil tac, but the marker CH03d01 of the gene Rvi9 has been found only in Gobustan selected variety. The marker OPB18 SCAR of the gene Rvi12 and the marker CH02c06 of the gene Rvi12 have been not found in any selected varieties. This results of both markers were the same as those of our study on the detection of resistance genes of introduced varieties (Khankishiyeva 2020) [25]. The marker CH04f03 of the gene Rvi13 has been found in Payizlig Guba and Sulh selected varieties. But the marker has been found only Aport introduced variety (Khankishiyeva 2020) [25]. The marker CH02b10 of the Rvi4 has been not found in any selected varieties. The results of marker were the same as those of our study on the detection of resistance genes of introduced varieties (Khankishiyeva 2020) [25]. The marker CH02b07 of the gene Rvi13 was found only in Gizil tac and Sulh selected varieties.This marker was not found in any of the introduced varieties in our other study (Khankishiyeva 2020) [25]. The marker CH-Vf1 of the gene Rvi6, the marker FMACH_VM2 and FMACH_VM3 of the gene Rvi5, the markers SSR-23.17 and SSR-24.19 of gene Rvi12, the marker Vr2C5'UTR of Rvi14, the marker Hi07h02 of gene Rvi5 have been not found in any selected varieties. This results of the above-mentioned markers were similar to the results of our

study on the detection of resistance genes of introduced varieties. This markers were not found in any of our introduced varieties (Khankishiyeva 2020) [25]. The CH02f06 was found in Dawamli, Sulh, Gizil tac, Azerbaijan, Gobustan, Elwin, Naile, Payizlig Guba selected varieties, but this marker was found in all of our introduced varieties. The CH02c02a of the Rvi4 gene has been found only in Sulh variety. But this marker for Rvi 4 scab resistance gene was not found introduced varieties (Khankishiyeva 2020) [25]. The OPL19SCAR marker of the gene Rvi2 has been found in Dawamli, Sulh, Azerbaijan, Gobustan, Elwin, Payizlig Guba, Naile, but did not detected in selected variety Gizil tac. This marker, in contrast to the selected varieties, also was found in all our introduced varieties in another study (Khankishiyeva 2020) [25].

Finally, we would like to note that the marker results for the scab disease resistance genes found in the donor varieties used in this study were identical to the results of our other research on the detection of the scab disease resistance genes in the introducted varieties.

Table 3: Screening the molecular markers of the scab resistance genes in apples

Varieties	Markers																						
	CH02c02a	**Hi07H02**	**Pl2_F/R**	**Hb09**	**OPL19**	**Fmach_vm3**	**Vr2C5'Utr**	**CH03d01**	**SSR24.91**	**CH02f06**	**SSR23.17**	**Fmach_vm3**	**CH-Vf1**	**CH02b07**	**At20 Scar**	**CH02b10**	**CH04f03**	**OPB18**	**CH02c06**	**CH05e03**	**CH02d12**	**Vg15 SSR**	**Vg12 SSR**	**Hi08e04**
Azerbaijan					+					+													+	
Naile					+					+														
Elwin					+					+													+	
Dawamli					+				+	+													+	
Payizlig Guba					+					+							+						+	
Sulh	+				+					+				+			+						+	
Gobustan					+			+		+													+	
Gizil tac									+	+				+						+			+	
Gala*			+																					
Golden delicious*																						+	+	
Priscilla*			+							+			+	+										
Antonovka*			+												+	+								
B45*			+		+															+				
TSR33T239*	+		+							+														
Durello di forli*			+											+					+					
Dülmener rosenapfel*			+	+										+										
9AR2T.196*		+	+																					
TSR34T.15*			+		+					+						+			+					
*Hansen`s baccata**			+						+	+						+			+					
*Malus baccata**			+					+														+		
A723.6*			+																					
J34*			+					+		+						+	+					+		
GMAL2473*	+		+							+														
Q71*			+																					+
Malus floribunda 821.*			+												+									
06005.55*			+														+							
06006.8*			+														+						+	
06006.57*			+														+						+	

* This donor varieties have also been used in our other research (Khankishiyeva 2020) [25]

It should be noted that the percentage of markers for resistance genes used against the pathogen *Venturia inaequalis* used in our study is, of course, related to their distances from the gene. For example, The results of molecular marker analysis in the table below show different ratios for both Rvi1-markers. The Vg12 SSR marker has been found in 53,3% of plants, and the Vg15SSR marker has been found in only 6,6 % of plants. This is due to the fact the Vg12 SSR marker is closer to the gene than the Vg15 SSR marker. The Vg12 SSR marker is only 0,12 cM away from the gene, while the Vg15 SSR marker is 0,14 cM away. Therefore, the interest rate of Vg12 SSR is estimated at 53,3%.

Also the results of molecular marker analysis show different ratios for both Rvi2-markers. The OPL19SCAR marker has been found in 70% of plants, and the CH02b10 marker has been found in only 3,3% of plants. This is due to the fact the OPL19SCAR marker is closer to the gene than the CH02b10 marker. The OPL19SCAR marker is only 1.0 cM away from the gene, while the CH02b10 marker is 8.0 cM away. Therefore, the interest rate of OPL 19 SCAR is estimated at 70%.

Table 4: Results of molecular marker analysis of apple genotypes used in our study

Markers	Number of markers	Ratio (%)
SSR-23.03 (Rvi12)	-	-
Rvi18-SSR (Rvi18)	-	-
Hi07h02 (Rvi5)	1	3,3
CH02c02a (Rvi4-Rvi15)	9	30.0
CH03c02 (Pld)	-	-
Pl2_F/R (Pl2)	20	66,6
HB09 (Rvi14)	1	3,3
OPL19SCAR (Rvi2-Rvi8)	21	70
FMACH_VM3 (Rvi5)	1	3,3
Vr2C5'UTR (Rvi4)	2	6,6
CH03d01(Rvi9-Rvi11)	4	13,3
SSR-24.91(Rvi12)	5	16,6
CH02f06 (Rvi15)	24	80
SSR-23.17 (Rvi12)	1	3,3
T6 (Rvi11)	-	-
NZmsCN943818 (Rvi16)	-	-
FMACH_Vm2 (Rvi5)	1	3,3
NH030a (Rvi16)	-	-
CH-Vf1 (Rvi17)	3	10
CH02b07 (Rvi13)	7	23,3
AT20SCAR (Pl1)	5	16,6
CH02b10 (Rvi2)	1	3,3
CH04f03 (Rvi13)	4	13,3
OPB18SCAR (Rvi8)	1	3,3
CH02c06 (Rvi12)	1	3,3
CH05e03 (Rvi11)	5	16,6
CH02d12 (Plm)	2	6,6
Vg15SSR (Rvi1)	2	6,6
Vg12SSR (Rvi1)	16	53,3
Hi08e04 (Rvi3)	3	10

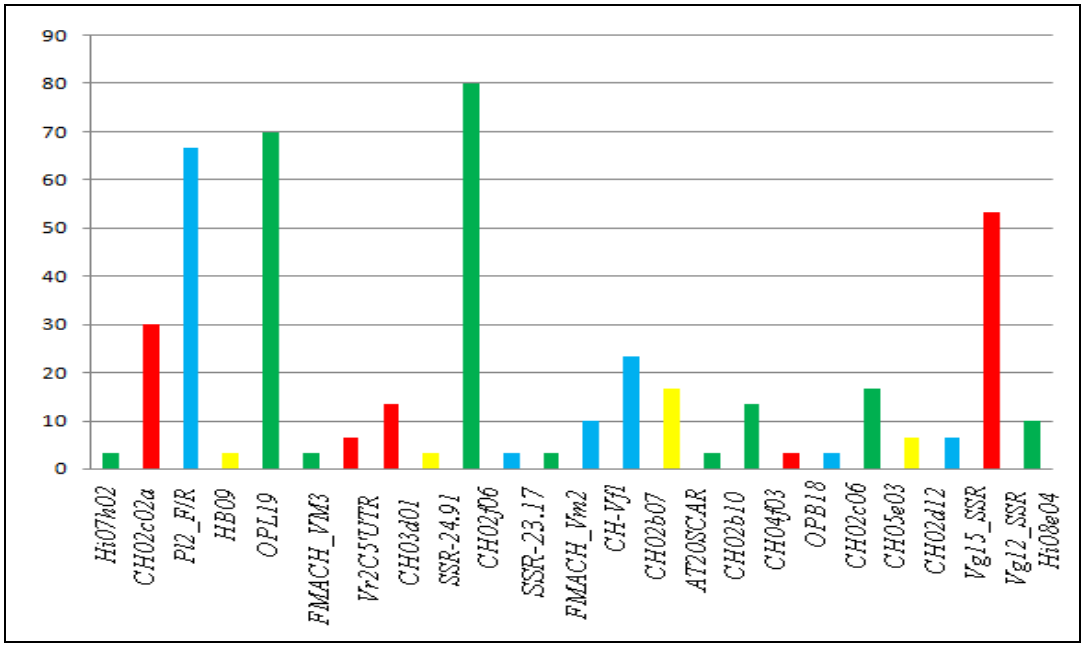

Fig 1: Percentages of molecular markers found in apple genotypes used in our study

Conclusion

As a result of this research, which was conducted for the first time in our country, for resistance genes against pathogen *Venturia inaequalis* were found in selection varieties of the Fruit and Tea-growing Research Institute. Selected apple varieties selected for their high genetic characteristics are expedient to be used as donor material in the expansion of private and state nursery farms of the our republic. We think that, can be used in breeding programs in the future from the new resistance varieties against scab.

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