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Selection of Central Asian apple species for scab resistance genes using molecular markers

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Abstract

Central Asia, one of the four apple origin regions in the world, has a significant diversity. The apple species in this region are particularly resistant to biotic and abiotic stress. Many of the mixed fruit forests, including apples, in Central Asia have been lost in the last half-century. It is emphasised that these forests can be preserved at the highest rate in mountainous Kyrgyzstan.

Scab (*Venturia inaequalis* (Cke.) Wint.), which is the most important apple disease in the world, causes a high level of production losses. Therefore, the best solution is to develop cultivars resistant to this disease. More than 20 major scab resistance genes have been identified in various cultivars and several wild relatives. In this study, four apple species found in Kyrgyzstan were screened using corresponding molecular markers for scab resistance. A total of 12 markers expressing 8 genes associated with scab resistance were used in the study. The 100 cultivars and genotypes used in the study produced bands for markers between 1–9. One genotype of *Malus niedzwetzkyana* species carried 7 of 8 resistance genes.

This study reveals that Kyrgyzstan apple germplasm is an important resource for scab resistance and breeding with marker-assisted selection.

Keywords: breeding, *Malus* spp., marker-assisted selection.

Introduction

The apple (*Malus domestica* Borkh.) is a fruit species that can be grown in all continents except Antarctica, in temperate climate regions and at high altitudes of tropical areas. This species is one of the most grown fruit species in the world, and its annual production is about 93 million tons. The largest producer is China, which produces about 46 million tons of apples. In Central Asian countries, the total production of apples is 1.83 million tons: Uzbekistan 1.2 million tons, Kazakhstan 262 thousand tons, Tajikistan 239 thousand tons, and Kyrgyzstan 136 thousand tons. One of the most important producing countries is Turkey with a production amount of 4.4 million tons (FAO, https://www.fao.org/faostat/en/#data/QCL).

It is known that the origin of cultivated apples has a complex structure. This complexity is demonstrated by a high level of cpDNA variation found in *Malus* cultivars studies (Coart et al., 2006). It was reported that wild species along the Silk Road: *M. baccata* (L.) (native to Siberia), *M. orientalis* (native to the Caucasus), and *M. sylvestris* Mill. (specific to Europe), may have contributed to the formation of cultivated apple trees. On the other hand, *M. sieversii* (Ldb.) M. Roem, a wild apple of Central Asian, is considered to be the main species contributing to the gene pool of *M. domestica* (Cornille et al., 2012).

As one of the homelands of the apple, Central Asia is an important origin of the apple and has a rich

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genetic diversity. Kyrgyzstan is one of the important apple gene centres in Central Asia. In different regions of the country, there are large natural apple populations along with other fruit species. It is emphasised that there are human-induced losses in these genetic resources, and this rich diversity may disappear in the future (Dzunusova et al., 2008). Mixed fruit forests in Central Asia have decreased significantly over the last 50 years, and only about 5–10% of forests have survived. It is emphasised that these fruit forests can remain at the highest level in Kyrgyzstan, the forest-rich country of Central Asia (Wilson et al., 2019).

The apple is a fruit that can be produced in different climates and is widely distributed throughout the world. This fruit, which is attractive to a wide audience of production and consumption, has diseases that negatively affect production. The most important disease that significantly affects commercial production is apple scab, caused by *Venturia inaequalis* (Cke.) Wint. This is a very common fungal disease affecting most of the apples grown in Australia, America, Asia, and Europe (Dunemann, Egerer, 2010). There is an intensive application of pesticides against apple scab today. The effects of these pesticides on the environment and human health as well as the cost have led researchers to pay more attention to this problem. This disease causes a decrease of total yield by up to 70% and great value losses (Jha et al., 2009). Although it varies with climatic conditions and cultivar resistance levels, the severity of damage particularly increases during periods of cool and rainy weather and in susceptible cultivars. In cases where chemical spraying is not applied, the economic losses reach up to 100% (Urbanovich, Kazlovskaya, 2008).

To achieve successful disease management, a well-integrated approach is usually needed. Selection of resistant or tolerant rootstocks and grafts (scion) cultivars, application of fungicides, biological disease control, improvement of the environment and selection of a suitable orchard site are the tools used to control apple scab disease (Belete, Boyraz, 2017). The most effective way to control the disease and reduce pesticide application is the use of resistant cultivars (Kaymak et al., 2013). Resistance to apple scab depends on the interaction of cultivar with a specific fungal population (Zelmene et al., 2022). Apple scab is a disease with a high genetic variability (Kaymak et al., 2016). The fungus undergoes genetic recombination every year increasing its ability to overcome host resistance.

In recent years, studies have been carried out to improve polygenic resistance, in which several different sources of resistance were combined. It is important to maintain longevity and consider the quality factors that determine apple resistance to black spot disease. Identification of polygenic sources, in which resistance is enhanced by major genes, is of great importance (Gessler et al., 2006). Therefore, several apple scab resistance genes were identified from wild apples, which were tried to be inserted into cultivated apples by classical breeding methods. The *Vf* (*Rvi6*) gene found in the 821 clone of *Malus floribunda* has been used as a source of resistance to black spot for many years. However, when the resistance of the *Vf* gene was broken in the 1980s, scientists turned to new research (Kaçal, Akinci Yildirim, 2011). Studies to date have identified at least 18 resistance genes associated with this disease (Khankishiyeva, 2020). On the other hand, more than 20 major scab resistance genes have been identified in various cultivars and a few wild relatives (Khajuria et al., 2018). The best solution to apple scab disease is to grow resistant cultivars, as the most of the commercial apple cultivars are susceptible to this disease. In this context, determining the resistance of the current apple genetic resources to this disease is a priority issue.

This study was carried out to identify apple scab disease resistance genes by molecular markers in four different apple species: *M. domestica, M. sieversii, M. kirghisorum*, and *M. niedzwetzkyana*, originating from Kyrgyzstan.

Material and methods

The research was conducted in 2021. The apple material used in the study consists of cultivars and genotypes collected from different regions of Kyrgyzstan. A total of 87 (44 for *M. domestica*, 20 for *M. sieversii*, 19 for *M. kirghisorum*, and 4 for *M. niedzwetzkyana*) genotypes belonging to four different apple species were used. Of this material, 56 were collected from Jalalabad, 16 from Issyk-Kul, 11 from Talas, and 4 from Cuy provinces. In general, these regions represent different apple-growing regions of Kyrgyzstan. These cultivars and genotypes are found in old orchards or mixed fruit forests in many regions of Kyrgyzstan. In addition to these, 4 Turkish ('Hüryemez', 'Sandık', 'Daldabir', and 'Amasya') and 9 foreign ('Red Chief', 'Idared', 'Elstar', 'Golden Delicious', 'Fuji', and 'Mutsu') and the standard ('Granny Smith', 'Royal Gala', and 'Starking Delicious') apple cultivars were included in the study (shown as genotype Nos. 88–100 in Table 2).

For DNA isolation, young leaves at the shoot tip of the plants were used. DNA isolations were performed using the CTAB protocol (Doyle, Doyle, 1990). For polymerase chain reaction (PCR) analysis, a total volume was 15 µl: 20 ng of template DNA, 1.5 µl of 10X buffer, 0.2 µM primer's, 2 mM $MgCl_2$, 0.3 mM dNTP, and 0.5 U Taq DNA polymerase, and the remaining volume was adjusted with distilled water.

A total of 18 primers including RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats), and SCAR (sequence characterised amplified region) were tested in the study, and all samples were tested with 12 markers, from which clear fragments were taken (Table 1).

Table 1. Primer list of scab resistance gene molecular markers used in the study

Resistance genes		Marker type	Marker name	Annealing temp. C°	Fragment (bp)	Reference
current	previous					
Rvi1	*Vg*	SSR	CH01d03	50	136–160	Liebhard, 2003
Rvi4	*Vh4*	SCAR	S22	55	1300	Hemmat et al., 2002
Rvi5	*Vm*	SSR	Hi07h02	66	230–277	Patocchi et al., 2009
Rvi6	*Vf*	SSR	CH-Vf1	64	166–180	Patocchi et al., 2009
		SCAR	AL-07	60	466–724	Tartarini et al., 1999
			ACS-9	63	469	Kaymak et al., 2013
Rvi8	*Vh2/Vh8*	SCAR	OPL19	62	430	Patocchi et al., 2009
Rvi11	*Vbj*	RAPD	OPB08	41	710	Gygax et al., 2004
Rvi12	*Vb*	SSR	Hi02d05	62	151–191	Padmarasu et al., 2014
			Hi07f01	60	207–210	Patocchi et al., 2009
		RAPD	B220	38	700	Erdin et al., 2006
Rvi15	*Vr2*	RAPD	OPK14	35	750	Kaymak et al., 2016

For RAPD analysis, 3 primers related to the scab resistance were used. The PCR cycle program was arranged as follows: one cycle of pre-denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 36°C for 45 s, primer extension at 72°C for 1 min, and a final extension at 72°C for 10 min. For 5 SSR primer pairs specific to SSR markers associated with scab resistance (Table 1), PCR cycles were performed as follows: one cycle of pre-denaturation at 94°C for 3 min, followed by 33 cycles of denaturation at 94°C for 40 s, annealing at 58°C for 40 s, primer extension at 72°C for 1 min, and a final extension at 72°C for 10 min. As for the analysis of SCAR markers associated with scab resistance, a similar PCR amplification profile was used for all 4 SCAR primer pairs specific to respective markers. PCR cycles were as follows: one cycle of pre-denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, primer extension at 72°C for 2 min, and a final extension at 72°C for 10 min.

PCR products were supplemented with 3 µl loading solution: 20 ml glycerol, 40%, 30 ml sterile water, and 0.05 g bromophenol blue. Resultant mixture was loaded onto 2.5% agarose gel and run under 115 V electric current for 2 h. For all electrophoresis processes, 5 µl 100 bp DNA ladder (Invitrogen, 15628019) was loaded onto agarose gel. After the electrophoresis process, the gels were transferred to the imaging device, the gel images were taken to the computer under UV light, and the bands were recorded.

Dendrograms were created to group apple material by the resistance gene. For this, fragments were evaluated and cluster analysis was performed based on the unweighted pair group method with arithmetic averages (UPGMA), and dendrograms were constructed using software NTSYS-pc 2.2 (Rohlf, 2009).

Results and discussion

A total of 12 markers representing 8 genes associated with scab resistance were used in the study. The 100 cultivars and genotypes used in the study produced bands for markers between 1–9 (Table 2). All of the material carried at least one scab resistance gene.

In terms of scab resistance, apple material has a great diversity. It was determined that genotype No. 23 of *Malus niedzwetzkyana* species carried 7 of 8 resistance genes used in the research. Only the band belonging to the S22 SCAR marker belonging to the *Rvi4* resistance gene could not be obtained. On the other hand, the same genotype did not produce bands in one of the 3 markers expressing the *Rvi6* resistance gene (ACS-9) and in one of the 3 markers expressing the *Rvi12* gene (Hi02d05); the remaining 9 markers were amplified from this genotype. In the study, 4 genotypes belonging to *M. niedzwetzkyana* species were used, and the other 3 genotypes Nos. 1, 2, and 14 were amplified for 3, 2, and 4 markers, respectively. In this regard, it was found that there is a significant variation in scab resistance within this species.

Similar results were obtained in previous studies. In a study of scab resistance of 23 *M. niedzwetzkyana* genotypes in Kazakhstan using OBP18 and OPL19 markers, some genotypes that carried resistance alleles for both markers were identified. All genotypes were resistant to the pathogen expressed by the OPL19 marker (Nurtaza et al., 2022). Similarly, 4 *M. niedzwetzkyana* genotypes used in the present study also carry the resistance allele for OPL19. This species is an essential component of the Central Asian mixed fruit forests. Researchers also noted that *M. niedzwetzkyana* was included in the Red Book of Threatened Species of Kazakhstan (Omasheva et al., 2015) and the Red List of Threatened Species (IUCN, 2016). Researchers emphasised that this species is found in minimal numbers in some areas of Kyrgyzstan and must be protected (Wilson et al., 2019).

Table 2. Apple species and cultivars tested for molecular markers associated with different scab resistance genes

Genotype No.	Species / cultivar	Collection site	*Rvi1*	*Rvi4*	*Rvi5*	*Rvi6*			*Rvi8*	*Rvi11*	*Rvi12*			*Rvi15*	Total
			CH01 d03	S22	Hi07 h02	CH-Vf1	AL-07	ACS-9	OPL 19	OPB 08	Hi02 d05	Hi07 f01	B 220	OPK 14	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*M. n.*	JA			1		1		1						3
2	*M. n.*	JA							1					1	2
3	*M. d.*	JA			1		1		1	1					4
4	*M. k.*	JA	1		1		1								3
5	*M. s.*	JA	1		1		1			1					4
6	*M. d.*	JA	1	1	1		1						1		5
7	*M. d.*	JA		1	1		1			1					4
8	*M. k.*	JA			1		1			1					3
9	*M. k.*	JA			1		1			1					3
10	*M. k.*	JA			1				1						2
11	*M. k.*	JA			1					1				1	3
12	*M. d.*	JA			1		1	1					1	1	5
13	*M. d.*	JA			1		1		1	1			1		5
14	*M. n.*	JA			1		1		1				1		4
15	*M. k.*	JA			1		1				1				3
16	*M. k.*	JA		1	1		1		1						4
17	*M. d.*	JA		1			1			1					3
18	*M. d.*	JA		1	1		1		1						4
19	*M. s.*	JA	1		1	1		1		1				1	6
20	*M. d.*	JA			1	1	1			1		1		1	6
21	*M. d.*	JA	1		1	1				1					4
22	*M. d.*	JA	1		1	1	1		1	1		1	1		8
23	*M. n.*	JA	1		1	1	1		1	1		1	1	1	9
24	*M. k.*	JA			1	1	1		1	1				1	6
25	*M. k.*	JA		1	1	1	1			1		1			6
26	*M. k.*	JA					1							1	2
27	*M. k.*	JA			1	1	1			1				1	5
28	*M. k.*	JA	1		1		1			1		1		1	6
29	*M. k.*	JA	1		1		1			1		1		1	6
30	*M. k.*	JA	1			1				1					3
31	*M. d.*	JA	1		1	1	1			1		1		1	7
32	*M. k.*	JA		1	1		1			1		1			5
33	*M. d.* Sary Chelek	JA			1	1	1		1			1		1	6
34	*M. d.* Zimny	JA	1		1	1	1			1		1		1	7
35	*M. d.*	JA			1		1		1						3
36	*M. d.* Kirgysky Zimny	JA			1		1	1	1	1		1			6
37	*M. d.*	JA	1		1		1								3
38	*M. d.* Suzak Sarısı	JA	1		1		1		1						4
39	*M. d.* Rashida	JA	1		1	1	1		1					1	6
40	*M. k.*	JA			1	1	1	1	1	1				1	7
41	*M. d.* Biskek	JA				1	1		1						3
42	*M. d.* Colpan	JA	1		1		1		1						4
43	*M. d.* Rozmarin	JA			1	1	1	1	1	1					6
44	*M. d.* Smirenko	JA			1	1	1			1				1	5
45	*M. d.* Aygul	JA			1										1
46	*M. d.* Sinap	JA			1		1			1					3
47	*M. d.* Alma Ata Aport	JA	1	1	1	1	1			1					6
48	*M. d.*	JA	1		1	1	1			1					5
49	*M. d.*	JA	1		1		1		1			1		1	6
50	*M. d.* Akalma	JA			1		1		1			1		1	5
51	*M. d.* Sabran	JA	1		1					1				1	4
52	*M. d.*	JA			1		1		1	1				1	5
53	*M. s.*	JA	1		1		1		1	1					5

Table 2 continued

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
54	*M. s.*	JA			1		1		1	1					4
55	*M. d.*	JA			1		1		1	1		1			5
56	*M. s.*	JA			1		1		1						3
57	*M. d.*	CU			1		1		1						3
58	*M. d.* Tomson	CU		1	1				1	1			1		5
59	*M. d.* Kirgysky	CU			1		1		1						3
60	*M. d.* Prevosh	CU			1	1	1		1						4
61	*M. s.*	IK			1			1	1						3
62	*M. s.*	IK			1	1	1		1			1			5
63	*M. s.*	IK			1										1
64	*M. s.*	IK	1		1	1	1		1	1					6
65	*M. s.*	IK		1	1		1			1					4
66	*M. d.* Tomson 2	IK			1		1		1						3
67	*M. d.* Delicious	IK			1		1	1							3
68	*M. d.* Saltanat	IK			1		1		1			1	1		5
69	*M. d.* Zailiysk	IK			1			1				1		1	4
70	*M. d.* Melba	IK		1	1	1	1			1					5
71	*M. d.* Almatinske grusovka	IK			1				1						2
72	*M. s.*	IK			1		1		1					1	4
73	*M. s.*	IK			1	1	1	1					1		5
74	*M. d.* Akalma	IK		1	1	1	1		1	1					6
75	*M. s.*	IK			1		1	1							3
76	*M. d.* Grusovka	IK			1	1	1								3
77	*M. d.*	TA			1	1									2
78	*M. s.*	TA			1		1			1					3
79	*M. s.*	TA		1	1		1				1			1	5
80	*M. s.*	TA		1	1	1			1	1				1	6
81	*M. s.*	TA	1		1				1	1					4
82	*M. s.*	TA			1	1			1						3
83	*M. k.*	TA		1	1				1	1				1	5
84	*M. k.*	TA			1	1			1						3
85	*M. k.*	TA			1										1
86	*M. s.*	TA			1				1	1					3
87	*M. s.*	TA				1			1	1	1				4
88	*M. d.* Red Chief	ERU			1				1	1			1		4
89	*M. d.* Idared	ERU			1					1			1		3
90	*M. d.* Elstar	ERU			1								1		2
91	*M. d.* Amasya	ERU		1	1								1		3
92	*M. d.* Daldabir	ERU			1	1			1				1		4
93	*M. d.* Golden Delicious	ERU			1						1		1	1	4
94	*M. d.* Sandık	ERU			1		1								2
95	*M. d.* Fuji	ERU			1				1				1	1	4
96	*M. d.* Mutsu	ERU			1		1					1	1	1	5
97	*M. d.* Granny Smith	ERU			1										1
98	*M. d.* Royal Gala	ERU			1								1		2
99	*M. d.* Hüryemez	ERU			1				1	1			1		4
100	*M. d.* Starking Delicious	ERU			1				1	1				1	4

M. n. – Malus niedzwetzkyana, *M. d. – Malus domestica*, *M. k. – Malus kirghisorum*, *M. s. – Malus sieversii*; JA – Jalalabad, Kyrgyzstan, CU – Cuy, Kyrgyzstan, IK – Issyk-Kul, Kyrgyzstan, TA – Talas, Kyrgyzstan, ERU – Erciyes University, Turkey

The species is a vital genetic resource for apple breeding and developing of new superior cultivars. Among the apple genotypes, No. 22 *M. domestica* has the same resistance genes as No. 23 *M. niedzwetzkyana*, except for the *Rvi15* resistance gene. In addition, 2 *M. domestica* (No. 45 'Aygul' and No. 97 'Granny Smith'), one *M. sieversii* (No. 63) and one *M. kirghisorum* (No. 85) genes are resistant to only one marker. There has been variation in carrying scab resistance genes within species. In addition to material with only one or a few resistance genes in species, there is also material with multiple genes.

The *Rvi1* resistance gene expressed by the CH01d03 marker was found in 23 apple genotypes used in the study. This gene was identified in 'Golden Delicious' and mapped at the bottom of LG12 (Durel et al., 2004; Khajuria et al., 2018). In the present study, the *Rvi4* resistance gene was identified in 16 apple accessions. This gene was one of the resistance genes detected in several apple materials. In the present study, regarding this gene, specific bands were achieved only in 'Amasya' among the standard cultivars.

The S22 SCAR marker was used to explain the putative presence of the *Rvi4* resistance gene, which was previously identified by a gene-for-gene approach in the F2 derivate TSR33T239 of Russian apple and *V. inaequalis* isolate J222 (Bus et al., 2005). It was found that the 1300 bp marker associated with the *Rvi4* gene was amplified using S22 SCAR marker at 4 cM on LG2 (Hemmat et al., 2003).

The *Rvi5* resistance gene was identified in 94 out of 100 apple materials during the study and was the most commonly detected resistance gene. Although apple genotypes have some resistance alleles, they may not be resistant to the disease. Because there are many alleles associated with resistance, one or more of them may not be sufficient to make a genotype resistant. For example, Papp et al. (2020) showed a 7 out of 9 disease symptom degree despite 'Golden Delicious' carrying the *Rvi1* resistance gene, and the SR33t239 apple genotype showed a 5th degree disease symptom despite carrying the *Rvi4* resistance gene. It has also been suggested that this resistance may be due to already described major genes or polygenic quantitative resistance. Hi07h02 SSR marker developed previously closely linked *Rvi5* gene on LG17 (Patocchi et al., 2005). On the other hand, the distal end of LG17 was mapped at 1 cM in the populations of 'Galaxy' × 'Murray' and 'Golden Delicious' × 'Murray' (Cova et al., 2015; Khajuria et al., 2018).

The presence of the *Rvi6* resistance gene in *Malus* species was screened using three different types of molecular markers including CH-Vf1 SSR, and AL-07 and ACS-9 SCAR ones. The CH-Vf1 marker was amplified from 32 of 100 apple accessions. The band of this marker was obtained in different genotypes of the four apple species used. A specific DNA region of 724 bp amplified with AL-07 SCAR marker linked to *Rvi6* gene in 67 apple genotypes; it was the second most common marker in apple species among the markers used in the study. On the other hand, in the present study, for the ACS-9 marker, specific 469 bp bands were achieved in only ten accessions. Although this band was not found in any genotype of *M. niedzwetzkyana*, it was obtained in some material of the other three species except standard cultivars (genotype Nos. 88–100). Of the 100 apple material used in the study, 80 were resistant to at least one marker. The *Rvi6* gene was identified in one *M. kirghisorum* (No. 40), one *M. domestica* (No. 43), and one *M. sieversii* (No. 73) with all three markers.

Genotypes of different species were used in the study. Previous studies did not find this subject belonging to the *M. kirghisorum* species. There are studies conducted with *M. sieversii* (Bus et al., 2005); however, in those studies, the *Vh8* (*Rvi8*), but not the *Rvi6* gene was used. *Rvi6* is the first scab resistance gene identified in a wild apple *M. floribunda* (Gessler et al., 2006). This gene remains the most extensively studied and characterised apple scab resistance gene.

In resistant segregants, the plant shows achlorotic-type reaction. Other types of reactions ranging from class 1 to 3b were observed in different progenies carrying the *Rvi6* resistance gene. This has been attributed to the associated QTL effect or the different genetic environment of the gene (Khajuria et al., 2018). The *Rvi8* resistance gene expressed by the OPL19 SCAR marker is a common gene among apple material. This gene was identified in *M. sieversii* and mapped on LG2 (Padmarasu et al., 2014). In the present study, 433 bp fragment was amplified. On the other hand, this fragment has been observed in some scab resistance cultivars such as 'Fuji'. Similar results were obtained in other studies (Khankishiyeva, 2020). The researchers detected fragments of the OPB19 marker in all species studied (Höfer et al., 2021). It was also determined that all 23 *M. niedzwetzkyana* genotypes contained a 430 bp resistance allele for the OPB19 marker (Hemmat et al., 2002). This suggests that various studies are needed on this issue.

The OBP08, one of the three markers developed linked to the *Rvi11* (formerly *Vbj*) resistance gene (Gygax et al., 2004), was detected in almost half of the material in the present study. Different genotypes of the four different apple species used showed resistance to this marker. Some standard cultivars ('Red Chief', 'Idared', 'Hüryemez', and 'Starking Delicious') also produced a corresponding fragment. These results revealed that this marker is not very suitable for the resistance of the *Rvi11* gene for the material used.

Three markers (Hi02d05, Hi07f01, and B220) used in the study are associated with the *Rvi12* resistance gene. Hi02d05 SSR was the least amplified of these markers in apple material. Only four apples (two *M. sieversii*,

No. 79 and No. 87, one *M. kirghisorum*, No. 15, and one *M. domestica* No. 93 'Golden Delicious') produced fragments associated with this marker. On the other hand, the *Rvi12* gene was detected in 18 apple accessions with the Hi07f01 SSR marker. Although this marker is more common in material belonging to *M. domestica* species, it was found only in 'Mutsu' among the standard cultivars. The third B220 marker expressing the *Rvi12* gene was detected in 19 apples, most of which belong to the *M. domestica* species. Only 2 *M. niedzwetzkyana* (No. 14 and No. 23) and one *M. sieversii* (No. 73) apples produced fragments associated with this marker. No apple belonging to the *M. kirghisorum* species produced fragments for this marker. On the other hand, one of the most common markers in standard cultivars was B220. The *Rvi12* gene was identified from the Siberian crab apple 'Hansen's baccata #2'. This gene was identified in LG12 between Hi02d05 and Hi07f01 SSR markers (Khajuria et al., 2018). Also, the $UBC220_{700}$ marker is closely associated with the *Rvi12* gene and mapped on LG1 (Hemmat et al., 2003).

The OPK14 marker expresses the *Rvi15* resistance gene used in the study. The band of this marker was obtained in 30 out of 100 apple materials used. Standard cultivars such as 'Golden Delicious', 'Fuji', 'Mutsu', and 'Starking Delicious' were also included. *Rvi15* gene is mapped on LG2 using the progeny of a cross between 'Idared' and GMAL 2473 (Khajuria et al., 2018).

The carrying conditions of scab resistance alleles of the genotypes of the species used in the study are presented in Table 3. In all species, the most common marker was the Hi07h02 associated with the *Rvi5* resistance gene, and the least common marker was the Hi02d05 associated with the *Rvi12* resistance gene. A band-producing marker was not found in any genotype of the species. The B220 marker did not produce bands in any of the 19 *M. kirghisorum* genotypes, while the S22, ACS-9, and Hi02d05 markers did not produce bands in any of the 4 *M. niedzwetzkyana* genotypes.

Numerous clusters were formed in the dendrogram, which was created based on the resistance genes carried by the apple accessions. In this regard, the similarity level of the materials varied from 0.27 to 1.00 (Figure).

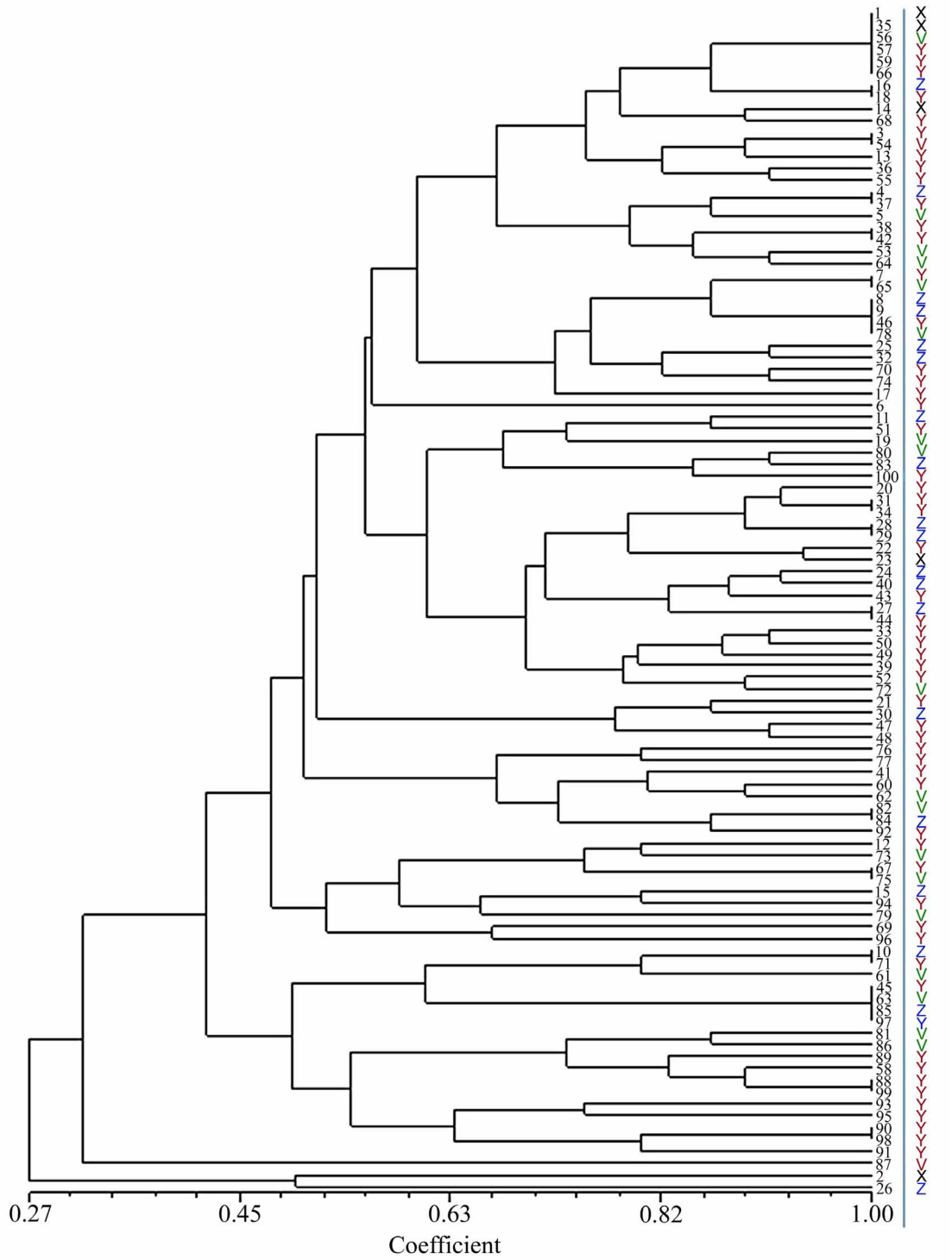
Table 3. Evaluation of the genotypes used in the study in terms of the presence of scab resistance genes on the basis of *Malus* species

Species	Genotype[1] No.	*Rvi1*	*Rvi4*	*Rvi5*	*Rvi6*		*Rvi8*	*Rvi11*	*Rvi12*			*Rvi15*
		CH01 d03	S22	Hi07 h02	CH-Vf1	AL07	ACS-9	OPL 19	OPB 08	Hi02 d05	Hi07 f01	B220	OPK 14
M. domestica	57	13	9	55	18	39	5	29	25	1	12	16	16
M. sieversii	20	5	3	19	7	12	4	12	11	2	1	1	4
M. kirghisorum	19	4	4	17	6	13	1	6	12	1	4	0	8
M. niedzwetzkyana	4	1	0	3	1	3	0	4	1	0	1	2	2
Total	100	23	16	94	32	67	10	51	49	4	18	19	30

[1] – belonging to the species used in the study

It was observed that the apples used in the study have a significant level of variation in the presence of the scab resistance gene. A previous study investigated the genetic diversity of some apple genotypes originating from Kyrgyzstan (Uzun et al., 2019). The materials used were genetically separated from each other and divided into numerous subgroups. In the current study, the genotypes *M. niedzwetzkyana* No. 2 and *M. kirghisorum* No. 26, which produced bands in the dendrogram for only two markers, differed from the other material. Additionally, the *M. sieversii* genotype No. 87 was loaded alone and did not cluster with any genotype.

The genotypes of the species are not clearly grouped in the dendrogram. No species-based segregation was identified. However, genotypes belonging to the species mostly clustered in small groups. Some genotypes of different species are similar, because they carry the same marker of the same resistance gene. Most of the standard cultivars (Nos. 88, 89, 90, 91, 93, 95, 98, and 99) are grouped, because they carry similar markers. The presence of a large number of small groups in the dendrogram indicates a high diversity level of the studied markers in the apple genotypes. Genotypes carrying the same resistance gene markers were generally placed into the same groups. On the other hand, some genotypes with a higher number of resistance genes (Nos, 23, 22, 20, 24, 28, 29, 31, and 34) shared the same subgroup. Six apple genotypes (Nos. 1, 35, 56, 57, 59, and 66) that produced fragments with the same resistance markers (Hi07h02, AL-07, and OPL19) were grouped as entirely similar. The dendrogram was suitable for showing which genotypes carry the same or similar resistance genes.



Note. Numbers in the Figure are given as genotype No. in Table 2; X – *M. niedzwetzkyana*, V – *M. sieversii*, Y – *M. domestica*, Z – *M. kirghisorum*.

Figure. Dendrogram showing the grouping of *Malus* spp. material according to the presence of resistance markers

Conclusions

1. This is the first comprehensive report on the presence of scab resistance genes in Kyrgyzstan apples.

2. The results revealed that apple species originating from Kyrgyzstan show significant differences in scab resistance genes. All accessions carried at least one scab resistance gene, and a significant variation of the resistance genes for the apple accessions was observed. Genotype No. 23 of the *Malus niedzwetzkyana* species carried 7 of the 8 resistance genes evaluated in the study.

3. Some genotypes of wild (No. 22 and No. 23) and cultivated (Nos. 22, 31, and 34) species were found to be a major source of resistance. These genotypes carry seven or more resistance markers. At the same time, it is very important to preserve and evaluate this endangered material. Controlled hybridisation between the resistant genotypes identified in the study and the cultivars with

good fruit quality will contribute to the development of new cultivars that are suitable for cultivation, have high commercial value, and are resistant to apple scab disease.

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