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RESEARCH PAPER

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MOLECULAR ANALYSIS OF SOME DISEASES AND REPRODUCTION CHARACTERISTICS IN APPLES FROM CENTRAL ANATOLIA (NIĞDE PROVINCE)

Orkun Gencer✉ https://orcid.org/0000-0001-6708-5772

Sedat Serçe https://orcid.org/0000-0003-4584-2028

Department of Agricultural Genetic Engineering, Faculty of Agricultural Science and Technologies, Niğde Ömer Halisdemir University, Niğde, Türkiye

ABSTRACT

Apple scab and fire blight are among the main diseases in apple production. Researchers are conducting studies to tackle these diseases as well as endeavoring to provide apple producers with disease-resistant plant materials. Self-incompatibility in apples engenders problems in pollination and yield. Molecular studies are crucial for revealing the potential of plant materials in this aspect. In this study, 48 genotypes among Niğde Misket Apple were investigated regarding apple scabs and fire blight resistance as well as self-incompatibility with respective markers and genes. Results showed genotypes had resistance alleles of *Rvi6* and QTL *FB_Mar12,* as well as the presence of S_{26} and S_9 alleles of the *S* gene. These results highlight new hypotheses for further research, particularly regarding disease resistance related to these genes, as well as the relationships among genotypes, cultivars, and species carrying these alleles.

Keywords: *Malus domestica*, plant genetic resources, *Venturia inaequalis*, *Erwinia amylovora*, DNA analysis, *S* alleles

INTRODUCTION

The cultivation of apples (*Malus domestica* Borhk.) spreads around the globe, where a temperate climate prevails. The center of origin of the apple is Central Asia, the Caucasus, and Anatolia (Türkiye) [Brite 2021]. Production of apple reached 95 835 965 tons globally, and Türkiye is one of the most important apple producers, ranked second with 4 817 500 tons [FAO 2024]. Niğde province is an important apple producer in Türkiye, and third in apple production with 581 304 tons [TÜİK 2024].

Disease of Apple scab is one of the most important fungal diseases that requires several fungicide applications. *Venturia inaequalis* is the fungus causing this disease. In most cases, up to 15 fungicide sprays are required to tackle the disease [MacHardy 1996]. Still, when cultivating vulnerable cultivars in some regions with heavy disease pressure and rainfall, 20 to 30 sprays would be necessary to decrease the spread and damage of the fungus [Ayer et al. 2019]. Integrated pest management techniques may help to reduce fungicide inputs for disease control. In addition to that, it is crucial to utilize supplementary disease management techniques to establish fungus-free orchards [Van Den Bosch et al. 2018]. Therefore, lowering the risk of fungicide breakdown and disease pressure is aided by leaf litter reduction [Porsche et al. 2017].

✉ orkungencer@gmail.com

If scab disease spreads and causes damage despite all efforts, cultivating scab-resistant apple varieties is inevitable as a last resort in apple production.

Most scab-resistant apple cultivars developed to date rely predominantly on a single resistance gene, *Rvi6* (*Vf*). The durability of this resistance is compromised, as virulent isolates (*avrRvi6*) have already been reported across Europe and in the USA [Vinatzer et al. 2004]. Moreover, for many of the scab resistance genes used in apple breeding, corresponding virulent isolates have been identified, with some isolates exhibiting multiple virulences [Peil et al. 2018]. Although current cultivars are still feasible for apple production to some degree in terms of this manner, these observations underscore the necessity of developing new cultivars with more durable and long-lasting scab resistance.

One of the main factor that limits apple output is fire blight, which is brought on by the necrotrophic bacteria *Erwinia amylovora* Burr. [Sobiczewski et al. 2017]. The polyphagous bacteria infest all aboveground organs of multiple host plant species, mostly those in the *Rosaceae* family [Zwet et al. 2012]. Infection frequently results in the rapid death of the infected parts or the whole plant. Environmental factors, plant susceptibility, and the size and appearance of the infection site all affect how severe the illness is. Apple trees are mostly protected from fire blight by combining chemical treatments with cultural applications; however, this does not always ensure complete efficacy. The majority of restrictions are linked to the pathogen's ability to survive on host plants, changes in environmental conditions, and insufficient bactericides [Peil et al. 2009]. Utilizing cultivars that are tolerant or resistant increases the likelihood that the disease will be less detrimental to the orchard's establishment [Sobiczewski et al. 2021].

It takes 13 to 17 years of study to create new apple cultivars using traditional breeding techniques [Sedov 2014]. In order to choose new cultivars and their pollinizers, the procedure starts with the selection of parents who possess desirable features. The gametophytic self-incompatibility (GSI) mechanism in *Malus* limits the range of potential parental pairings [Pereira-Lorenzo et al. 2018]. The incapacity of a fertile plant to generate zygotes following self-pollination or pollination with pollen who share S-alleles is known as its GSI. Located on the 17th chromosome of the apple genome, the *S*-locus is in charge of determining self-incompatibility [Janssens et al. 1995, Sakurai et al. 2000]. To enhance genetic diversity in plant populations, genetically compatible crosses should be performed, including those involving different species when feasible. For this purpose, identifying the *S*-alleles of the genotypes within the population is essential when planning crosses [Karataş et al. 2023].

In the past, pollination and pollen tube growth tests were used to indirectly determine the presence of *S*-alleles. However, this approach is highly sensitive to environmental factors in various vegetative and generative periods in seasons to guarantee the accuracy of this determination [Muñoz-Sanz et al. 2020]. Breeders can design crosses between compatible genotypes by using genetic markers, such as allele-specific primers, to discover *S*-alleles and learn about their distribution among apple genotypes.

Niğde Misket Apple is the local apple landrace that produced since the early years of the Türkiye Republic around the Niğde province in Central Anatolia. According to morphological, pomological, and genetic analysis, Niğde Misket Apple shows a genetic diversity. Genetic analysis indicated similarity rates differentiate 0.61–1.00 [Gencer and Serçe 2022]. According to observations in the region, these local apple genotypes have also not been severely affected by apple scab as well as fire blight diseases compared to known cultivars in the same region, in spite of cultural practices not being carried out in a proper way and time mostly for orchards where Niğde Misket Apple produce.

Molecular studies are an important aspect of conducting modern studies on apples [Cieślińska and Borisova 2019] on many related topics, such as rootstocks [Stachowiak and Świerczyński 2012] and artificial intelligence [Ropelewska and Lewandowski 2024].

One of the biggest QTLs governing resistance to fire blight in apple is QTL *FBF7*. It was located on the Fiesta variety's 7th chromosome [Papp et al. 2015]. Previous research indicated the 210 bp allele of the CH-F7-Fb1 marker is linked to QTL *FBF7* fire blight resistance, and QTL *FBF7* is present in various apple cultivars such as Gala (174 bp allele that is not related to resistance) and Fuji (210 bp allele that resistant resistant-related) [Lyzhin and Saveleva 2021].

QTL *Fb_MR5* identified in *Malus* × *robusta* 5 on LG 3 [Peil et al. 2007]. The Ch03g07 molecular mark-

er was previously utilized to detect the presence of QTL *Fb_MR5* in a study, a 145 bp allele affiliated with resistance to fire blight of *QTL Fb_MR5* [Fahrentrapp et al. 2013].

QTL *FB_Mar12*, that a major effect on fire blight resistance, colocalized on the distal end of LG12 (linkage group 12) in *M. floribunda*, *M. Evereste,* and *M.* × *arnoldiana*, and findings of studies indicated QTL *FB_Mar12* potentially present genes with domains definitive to resistance of the disease [Durel et al. 2009, Emeriewen et al. 2017, 2021]. Although there is evidence of hybridization between these two species, it is still unknown if the resistance of fire blight QTL is independent or shared by both species [Emeriewen et al. 2021, Tegtmeier et al. 2023]. Previous studies detected QTL *FB_Mar12* with ChFbE01 in various species. ChFbE01 is affiliated only 266 bp size allele [Parravicini et al. 2011] for resistance.

QTL *Fb_Mfu10* is an important QTL for resistance to fire blight, which was identified on LG10 of *Malus fusca*, accession MAL0045 [Emeriewen et al. 2014, 2018, 2020, 2022, Mansfeld et al. 2023]. FRM4 molecular marker utilized to detect a resistance-related allele (156 bp) on this QTL [Emeriewen et al. 2014, 2018].

Rvi6 was the most prevalent *R* gene when considering all other scab-resistant cultivars from the gene bank and the first scab-resistance gene discovered from a wild cousin of apples (*M. floribunda* Siebold ex Van Houtte). This gene, which is found on LG1, is still widely researched and described as a gene for resistance to scab in apples [Vinatzer et al. 2004]. The resistance allele of the Ch-Vf1 marker showed a linkage with the Vf2ARD 527-bp fragment [Boudichevskaia et al. 2009] that is a resistant candidate gene.

The gene of *Rvi11* was discovered in *M. baccata* and was mapped to LG2 [Dayton and Williams 1968, Gessler et al. 2006]. CH02c06 marker was utilized previously, amplified various alleles (230, 236, 240, 248 bp), and the 248 bp allele were indicated as resistance-related [Gianfranceschi et al. 1998, Gygax et al. 2004]. A resistance allele of the gene was previously reported in the Modi cultivar [Madenova et al. 2024].

Rvi4 (*Rvi15*) was mapped on the top of LG 2 using Idared × GMAL 2473 population [Patocchi et al. 2004]. A recent study found that *Rvi4* and *Rvi15* are identical genes, and they suggested the use *Rvi4* name instead of *Rvi15* for future studies [Peil et al. 2023]. CH02f06 was utilized in studies to detect alleles of *Rvi4* (*Rvi15*) related to scab resistance at 146 bp [Galli et al. 2010, Patocchi et al. 2004], 152 bp [Patocchi et al. 2009], and 155 bp [Peil et al. 2023] on GMAL 2473. So, it's a compound repeat-type marker [Gianfranceschi et al. 1998]. Studies conducted on *Rvi4* (*Rvi15*) indicated that some known apple cultivars such as Gala, Granny Smith, Fuji, SuperChief, Modi, Pink Lady, Granny Smith, Honeycrisp and Jeromine were the carriers of *Rvi4* (*Rvi15*) resistance [Khankishiyeva 2020, Madenova et al. 2024].

The *Rvi5* gene was indicated to play a role in resistance in *Malus micromalus* Mak. and *Malus atrosanguinea* 804 [Dayton and Williams 1970]. A study on *Rvi5* developed the Hi07h02 (SSR marker), which is tightly linked with *Rvi5* on LG-17 [Patocchi et al. 2005]. A 228 kb area that most likely contains the *Rvi5* gene was recently discovered using the genome of the apple as a basis [Bandara et al. 2013]. Previous studies showed some apple cultivars indicated as carriers of the *Rvi5* gene resistance, such as Jeromine and SuperChief [Madenova et al. 2024]. Hi07h02 marker previously utilized and amplified 224 bp [Cova et al. 2015] and 230 bp [Patocchi et al., 2009] alleles coupling with the resistance in the *Rvi5* gene.

The *Rvi12* locus of scab resistance was identified from *M. baccata* Hansen's baccata #2 (HB2) [Dayton and Williams 1968]. *Rvi12* was mapped to LG 12 of the apple genome [Erdin et al. 2006]. Then a study on a fine map of the *Rvi12* locus of scab resistance indicated the scab resistance gene *Rvi12* from HB2 was reported on LG12 in the cross Gala × HB2, as mapping to the apple [Padmarasu et al. 2014].

Apple has a multi-allelic gametophytic incompatibility system controlled by a single *S* gene. Many alleles of the *S* gene are identified with different molecular markers [Janssens et al. 1995, Sakurai et al. 2000]. The MalusS_{26} marker can detect the S_{26} allele of the *S* gene on a 193 bp size [Sakurai et al. 2000]. This allele is a rare presence in known apple cultivars. Some examples of known S_{26} allele carriers are crabapple cultivar Baskatong and *Malus floribunda* 821, which has an apple scab resistance [Broothaerts et al. 2004], as well as local apple genotypes in Türkiye [Karataş et al. 2023], and Marubakaido apple rootstock (*Malus prunifolia* Borkh) [Brancher et al. 2020].

The MalusS$_9$ marker can detect the S_9 allele of the *S* gene on a 343 bp size [Janssens et al. 1995]. This allele is present among known apple cultivars like Starking Delicious, Fuji, and Jonagold [Broothaerts et al. 2004].

This study aimed to investigate apple scab and fire blight resistance/tolerance as well as the self-incompatibility status of Niğde Misket Apple genotypes with molecular markers. Central Anatolia, Türkiye, is already one of the major locations for apple production and the center of origin of apples in the world. Niğde Misket Apple has the potential for a future breeding program. This potential will be evaluated in terms of apple scab and fire blight resistance/tolerance and self-incompatibility, and shed light on future studies.

MATERIAL AND METHOD

Plant material. In this study, 48 different apple genotypes were used. These genotypes were collected from different areas in Niğde (Fig. 1, Table 1) [Gencer and Serçe 2022] and preserved in Niğde Ömer Halisdemir University Faculty of Agricultural Science and Technologies Faculty, as a collection orchard. All of them were grafted on MM106 apple rootstock with bud graft in 2019 and planted in the orchard in 2020. Leaves are collected from the collection orchard.

Fuji, Amasya, G41, Modi, Gala, SuperChief, and Golden Delicious cultivars were used as control groups for molecular analysis.

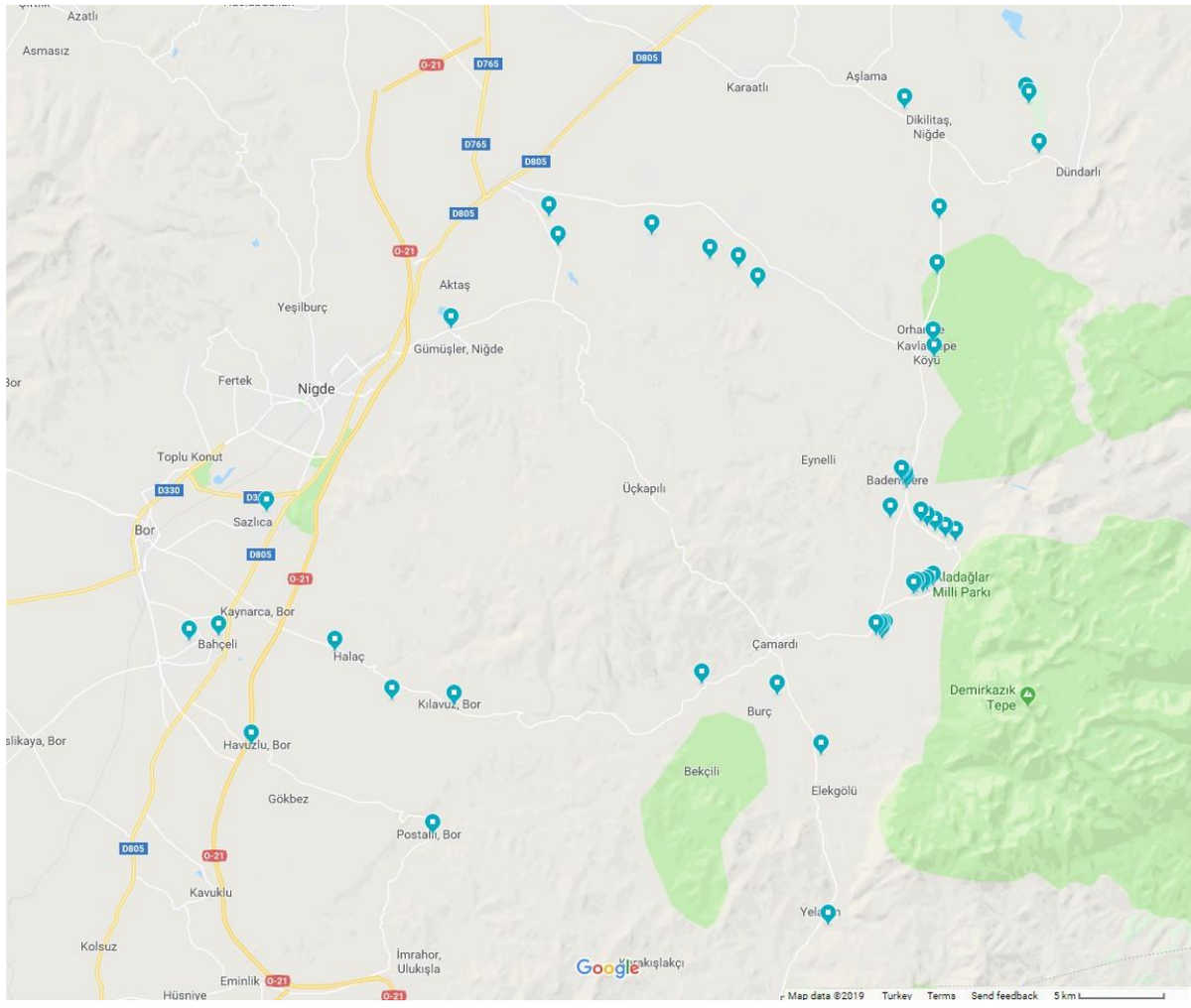

Fig. 1. Map of the collected genotypes. Blue markers indicated the GPS data [Gencer and Serçe 2022]

Table 1. Information related to the plant material [Gencer and Serçe 2022]

Tree codes	Name of locations	GPS data	Elevation (meter)
KMR	Kemerhisar	37°49'56.9"N 34°35'29.3"E	1125
BHC	Bahçeli	37°50'06.7"N 34°36'39.5"E	1147
SZL	Sazlıca	37°54'04.3"N 34°38'34.8"E	1211
HLC	Halaç	37°49'39.0"N 34°41'19.3"E	1297
KRC	Karacaören	37°48'04.1"N 34°43'36.9"E	1487
KLV	Kılavuz	37°47'53.8"N 34°46'06.7"E	1571
HVZ	Havuzlu	37°46'38.0"N 34°37'59.1"E	1213
PST	Postallı	37°43'46.9"N 34°45'17.0"E	1394
DGR	Değirmenli	38°02'54.4"N 34°54'06.4"E	1494
DND	Dündarlı	38°05'28.7"N 35°09'54.4"E	1326
CKR1	Çukurbağ	37°50'09.6"N 35°03'25.8"E	1484
CKR2	Çukurbağ	37°50'08.7"N 35°03'33.2"E	1493
CKR3	Çukurbağ	37°49'60.0"N 35°03'27.7"E	1499
CKR4	Çukurbağ	37°50'07.1"N 35°03'21.4"E	1480
CKR5	Çukurbağ	37°50'07.2"N 35°03'10.9"E	1455
BDM1	Bademdere	37°55'04.7"N 35°04'14.8"E	1601
BDM2	Bademdere	37°55'01.5"N 35°04'18.1"E	1595
BDM3	Bademdere	37°54'58.9"N 35°04'24.5"E	1586
BDM4	Bademdere	37°54'53.9"N 35°04'24.2"E	1582
BDM5	Bademdere	37°54'47.8"N 35°04'26.2"E	1576
PNR1	Pınarbaşı	37°53'43.7"N 35°05'00.8"E	1574
PNR2	Pınarbaşı	37°53'36.7"N 35°05'15.9"E	1569
PNR3	Pınarbaşı	37°53'26.4"N 35°05'35.5"E	1572
PNR4	Pınarbaşı	37°53'15.0"N 35°06'02.0"E	1562
DMR1	Demirkazık	37°51'41.0"N 35°05'31.5"E	1577
PNR5	Pınarbaşı	37°53'06.4"N 35°06'24.2"E	1598
DMR2	Demirkazık	37°51'32.2"N 35°05'16.6"E	1558
DMR3	Demirkazık	37°51'28.7"N 35°05'04.8"E	1545
DMR4	Demirkazık	37°51'28.4"N 35°04'50.9"E	1556
DMR5	Demirkazık	37°51'25.4"N 35°04'43.4"E	1560
CLL	Celaller	37°48'34.6"N 34°56'09.5"E	1687
BRC	Burç	37°48'12.9"N 34°59'11.4"E	1445
ELG	Elekgölü	37°46'18.5"N 35°00'59.3"E	1365
KVL1	Kavlaktepe	37°59'29.8"N 35°05'34.0"E	1671
KVL2	Kavlaktepe	37°59'00.8"N 35°05'34.9"E	1726
HCB1	Hacıbeyli	38°07'17.7"N 35°09'19.9"E	1280
HCB2	Hacıbeyli	38°07'05.3"N 35°09'28.9"E	1283
DKL	Dikilitaş	38°06'56.9"N 35°04'25.3"E	1435
YSL	Yeşilova	38°03'31.3"N 34°49'58.3"E	1388
ULG	Uluağaç	38°02'34.6"N 34°50'20.2"E	1435
GMS	Gümüşler	37°59'56.2"N 34°45'59.7"E	1344
HMM	Himmetli	38°02'08.8"N 34°56'32.7"E	1552
ELM1	Elmalı	38°01'52.1"N 34°57'41.6"E	1603
ELM2	Elmalı	38°01'12.8"N 34°58'29.0"E	1605
KCP	Kocapınar	38°01'37.2"N 35°05'43.2"E	1571
EYN	Eynelli	37°53'51.3"N 35°03'46.9"E	1531
ICM	İçmeli	38°03'24.2"N 35°05'49.6"E	1519
YLT	Yelatan	37°40'51.6"N 35°01'14.0"E	1320

Table 2. Information about genes / QTLs and markers

No	Markers	Sequence 5' to 3'	Gene/QTL	Chromo-some	Disease/Trait	Positive control	Negative control	Ta °C	Expected allele size*	References
1	CH-F7-Fb1_F	AGCCAGATCACATGTTTTCATC	QTL *FbF7*	7	fire blight	G41	Gala	57	174–**210** bp	[Lyzhin and Saveleva 2021, Papp et al. 2015]
	CH-F7-Fb1_R	ACAACGGCCACCAGTTTATC								
2	CH-Vf1_F	ATCACCACCAGCAGCAAAG	*Rvi6*	1	apple scab	Modi	Gala	60	129–180 bp 139, 166, **159** bp	[Boudichevskaia et al. 2009, Höfer et al. 2021, Madenova et al. 2024, Vinatzer et al. 2004]
	CH-Vf1_R	CATACAAATCAAAGCACAACCC								
3	CH02c06_F	TGACGAAATCCACTACTAATGCA	*Rvi11*	2	apple scab	Modi	Fuji	60	216–254 bp **248** bp	[Dayton and Williams 1968, Gessler et al. 2006, Gianfranceschi et al. 1998, Madenova et al. 2024]
	CH02c06_R	GATTGCGCGCTTTTTAACAT								
4	CH02f06_F	CCCTCTTCAGACCTGCATATG	*Rvi4* (*Rvi15*)	2	apple scab	Modi	Golden Delicious	60	**146**–158 bp **152**, **155**	[Galli et al. 2010, Gianfranceschi et al. 1998, Khankishiyeva 2020, Madenova et al. 2024, Patocchi et al. 2004, 2009, Peil et al. 2023]
	CH02f06_R	ACTGTTTCCAAGCGCTCAGG								
5	Ch03g07_F	AATAAGCATTCAAAGCAATCCG	QTL *Fb_MR5*	3	fire blight	G41	Fuji	60	119–171 bp **145 bp**	[Fahrentrapp et al. 2013, Peil et al. 2007]
	Ch03g07_R	TTTTTCCAAATCGAGTTTCGTT								
6	ChFbE01_F	TTCAAGTCCCTGCATTTCAC	QTL *FB_Mar12*	12	fire blight	G41	Golden Delicious	60	**266** bp	[Durel et al. 2009, Emeriewen et al. 2017, 2021, Parravicini et al. 2011, Tegtmeier et al. 2023]
	ChFbE01_R	CAAGCTCATTGACCAGTTCG								
7	FRM4_F	GGGTTTGGTGGAGTGTCAAT	QTL *Fb_Mfu10*	10	fire blight	G41 (not have resistance allele)	Fuji	60	**156**–166 bp	[Emeriewen et al. 2014, 2018, 2020, 2022, Mansfeld et al. 2023]
	FRM4_R	AAAGGCAGATCTGGTGATGC								
8	Hi07h02_F	ATTTGGGGTTTCAACAATGG	*Rvi5*	17	apple scab	SuperChief	Fuji	60	220–280 bp **224**, **230** bp	[Bandara et al. 2013, Cova et al. 2015, Dayton and Williams 1970, Madenova et al. 2024, Patocchi et al. 2005, 2009]
	Hi07h02_R	GTTTCGGACATCAAACAAATGTGC								
9	MalusS26_F	GAAGATGCCATACGCAATGG	*S*	17	self-incom-patibility	Amasya	Golden Delicious	55	193 bp	[Brancher et al. 2020, Broothaerts et al. 2004, Karataş et al. 2023, Sakurai et al. 2000]
	MalusS26_R	ATGAATTCTTAATACCGAATATTGGCC								
10	MalusS9_F	CAGCCGGCTGTCTGCCACTT	*S*	17	self-incom-patibility	Fuji	Golden Delicious	62	343 bp	[Brancher et al. 2020, Broothaerts et al. 2004, Janssens et al. 1995, Karataş et al. 2023]
	MalusS9_R	CGGTTCGATCGAGTACGTTG								
11	SSR-MDC005174.220_F	GTAGTAATCCACCCCCATGC	*Rvi12*	12	apple scab	Gala (not have resistance allele)	Fuji	60	209–223 bp **216** bp	[Dayton and Williams 1968, Erdin et al. 2006, Padmarasu et al. 2014]
	SSR-MDC005174.220_R	TGTATGACTCGTCGCTCACG								

* Bolded allele sizes are related to disease resistance according to references.

Investigation of fire blight resistance. For the investigation of fire blight resistance, the CH-F7-Fb1 marker targeted QTL *FbF7*, the Ch03g07 marker targeted to QTL *Fb_MR5*, the ChFbE01 marker targeted QTL *FB_Mar12*, and the FRM4 marker targeted to QTL *Fb_Mfu10* utilized in this study. These markers were selected because they have been validated in previous studies, and their expected allele sizes are suitable for detection using agarose gel. Details for markers (sequences, controls, Ta °C, expected allele sizes, and references) are given in Table 2.

Investigation of apple scab resistance. For the investigation of apple scab resistance, the CH-Vf1 marker targeted the *Rvi6* gene, the CH02c06 marker targeted the *Rvi11* gene, the CH02f06 marker targeted the *Rvi4* (*Rvi15*) gene, the Hi07h02 marker targeted the *Rvi5* gene, and the SSR-MDC005174.220 marker targeted the *Rvi12* gene utilized in this study. These markers were selected because they have been validated in previous studies, and their expected allele sizes are suitable for detection using agarose gel. Details for markers (sequences, controls, Ta °C, expected allele sizes, and references) are given in Table 2.

Investigation of self-incompatibility. For the investigation of self-incompatibility, MalusS_{26} and MalusS_9 markers targeted the *S* gene utilized in this study. MalusS_{26} and MalusS_9 markers were utilized due to the amplified alleles (S_{26} and S_9 alleles), with these markers being common among apples local to Anatolia [Karataş et al. 2023]. Details for markers (sequences, controls, Ta °C, expected allele sizes, and references) are given in Table 2.

DNA extraction. Extraction processes of DNA from healthy and young leaves were performed by the CTAB method [Dellaborta et al. 1983]. DNA concentrations were then analyzed by the Quawell Q5000 UV-Vis Spectrophotometer then diluted to 5 ng/µL.

Polymerase Chain Reaction steps. PCR was performed in 25 µL total volume with 5 ng DNA, 2.5 µL 10x buffer, 0.2 mM dNTPs, 0.5 µM of each primer, 2.0 mM $MgCl_2$, 1 U Taq DNA polymerase, and dH_2O. Thermal cycler protocol was performed following initial denaturation at 94 °C for 2 min, 35 cycles (1 min 94 °C, 1 min annealing temperature of primer, 1 min 30 s 72 °C), and final extension at 72 °C for 1 min.

Agarose gel conditions and evaluation. Agarose gel (2.5%) electrophoresis (110 volts, 2 hours 30 min) was used to separate the DNA fragments of different sizes in the PCR products. TriTrack DNA Loading Dye (6x) and The GeneRuler 50 bp DNA ladder (Thermo Fisher Scientific) were used to identify the sizes of DNA samples on gels. Gels are imaged utilizing an agarose gel imaging system (DNR MiniLumi Bio Imaging System). Positive controls always load on the gel as the first sample after the ladder. Negative controls always load on the gel last after all samples are loaded. A binary number file was created from the scored gel images (0 or 1 depending on the presence of bands on the gel).

Fragment analysis. Fragment analysis was conducted with primers (CH-Vf1 and ChFbE01) that showed resistance-related allele presence according to agarose gel results. PCRs were performed again with these primers and M13 primer (5′-CACGACGTTGTAAAACGAC-3′) to forward primers. Genotypes ICM and KMR are utilized in fragment analysis as plant materials because these two genotypes have clear and the same bands as the other genotypes that have resistance-related allele presence. 6-FAM and HEX fluorescent dyes are utilized to label M13 primers. Products are loaded to the Applied Biosystems (ABI) 3500 Series Genetic Analyzer with GeneScan™ 500 LIZ™ dye as a size standard, and results are evaluated in GeneScan® Analysis Software.

RESULTS

With fire blight-related markers. The results of the CH-F7-Fb1 marker, targeted to QTL FBF7, indicated no disease-related allele among the genotypes.

The results of the Ch03g07 marker, targeted to QTL Fb_MR5, indicated no disease-related allele among the genotypes.

The result of the ChFbE01 marker (Figs 4 and 5) targeted to QTL *FB_Mar12* indicated that 47 of the 48 genotypes (except BDM4) have a disease resistance-related allele (266 bp).

The result of the FRM 4 marker targeted to QTL *Fb_Mfu10* indicated no disease-related allele among genotypes.

With apple scab-related markers. The results of the CH-Vf1 marker targeted to the *Rvi6* gene (Figs 2 and 3) indicated that all genotypes have a disease resistance-related allele (159 bp). Except for genotype

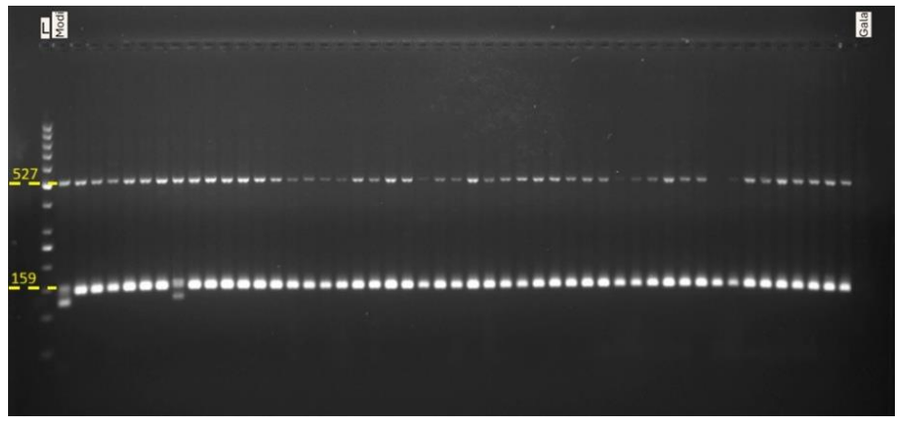

Fig. 2. Gel image of CH-Vf1 marker, yellow lines, and labels represent bp size of interest

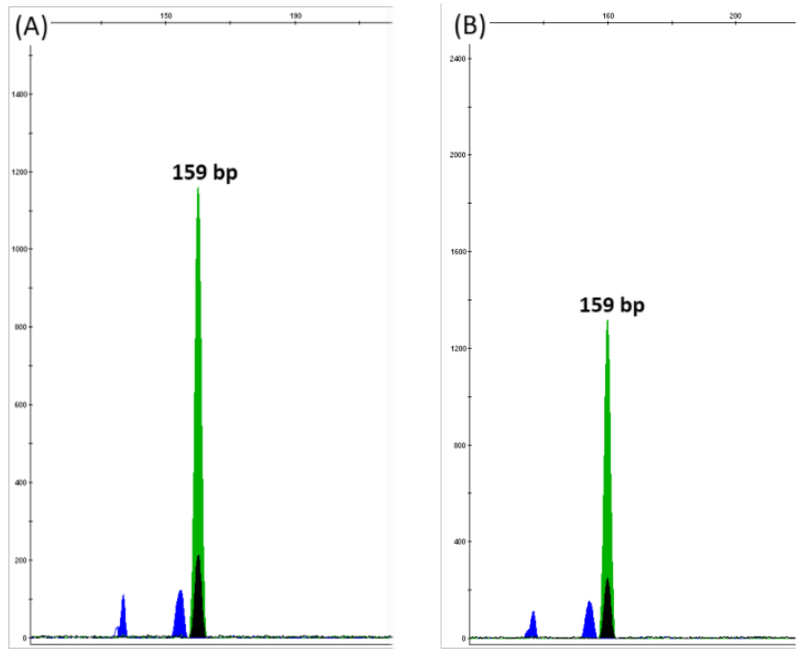

Fig. 3. Results of fragment analysis of CH-Vf1 marker (*Rvi6*). A) genotype ICM, B) genotype KMR

DMR4, other genotypes also have a 527-bp allele, which shows linkage to the 159 bp allele.

The result of the CH02c06 marker targeted to the *Rvi11* gene indicated no disease-related allele among genotypes.

The results of the CH02f06 marker targeted to the *Rvi4* (*Rvi15*) gene indicated no disease-related allele among the genotypes.

The results of the Hi07h02 marker targeted to the *Rvi5* gene indicated no disease-related allele among the genotypes.

The result of the SSR-MDC005174.220 marker targeted to the *Rvi12* indicated no disease-related allele among genotypes. The PIC value of the SSR--MDC005174.220 marker is 0.369.

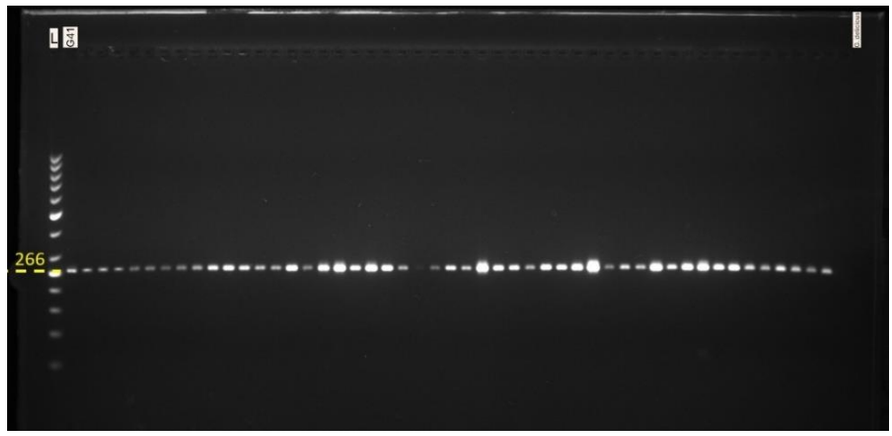

Fig. 4. Gel image of ChFbE01 marker, yellow line, and label represent bp size of interest

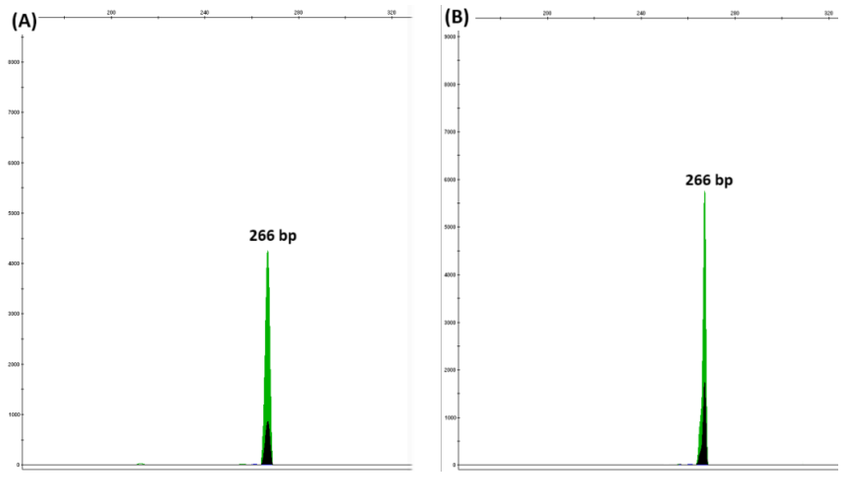

Fig. 5. Results of fragment analysis ChFbE01 marker (QTL *Fb_Mar12*). A) genotype ICM, B) genotype KMR

With self-incompatibility-related markers. The result of the MalusS_{26} marker targeted to the *S* gene (Fig. 6) indicated that 47 of the 48 genotypes (except the genotype PNR2) have S_{26} allele (193 bp) of the *S* gene.

The result of the MalusS_9 marker targeted to the *S* gene (Fig. 7) indicated that all genotypes have S_9 allele (343 bp) of the *S* gene.

DISCUSSION

According to the results, no disease resistance-related alleles presence detected with the utilized molecular markers (Table 2) for QTL *FBF7*, *Rvi11*, *Rvi4* (*Rvi15*), QTL *Fb_MR5*, QTL *Fb_Mfu10,* and *Rvi5* among the genotypes. Although other alleles were detected with some of these markers, the genetic variations already presented in the previous study with IPBS markers [Gencer and Serçe 2022]. Due to that fact, no further analysis was conducted for that purpose.

Results indicated resistance allele presence among the Niğde Misket Apple genotypes for *Rvi6* and QTL *FB_Mar12* with their respective molecular markers (Table 2).

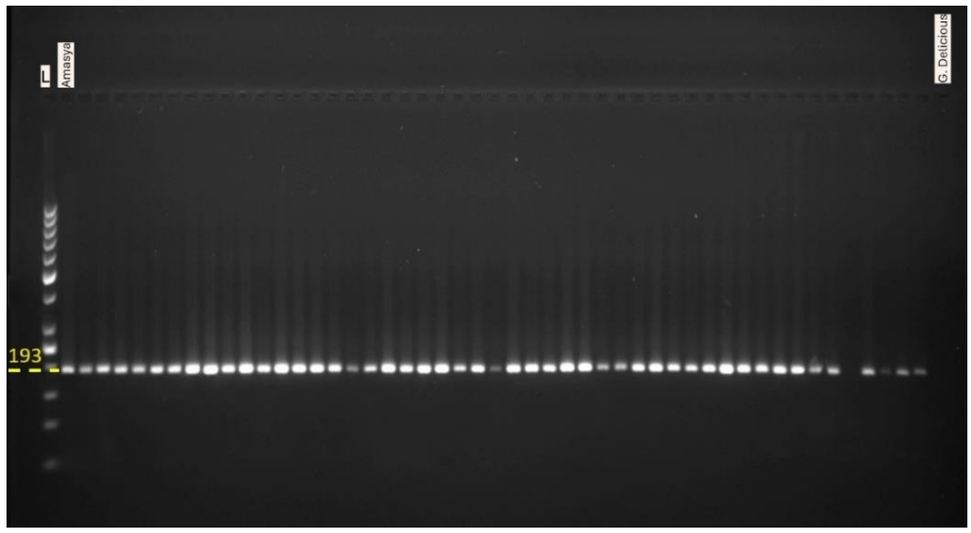

Fig. 6. Gel image of MalusS26 marker, yellow line, and label represent bp size of interest

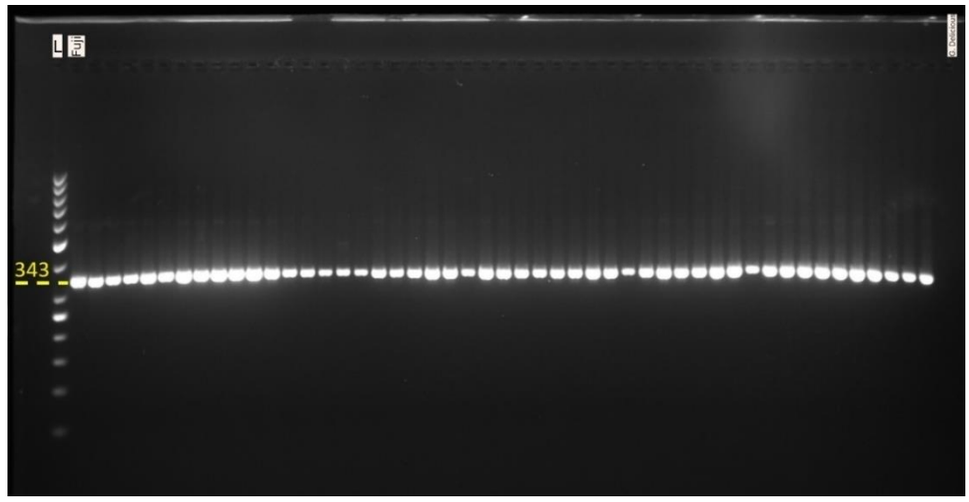

Fig. 7. Gel image of MalusS_9 marker, yellow line, and label represent bp size of interest

These results may fitted in due to *Rvi6* being the most abundant gene among *R* genes for scab resistance [Vinatzer et al. 2004] but given the limited scope of this study, it's not an exact proof. Also, the presence of the *Rvi6* has already been reported in different cultivars [Höfer et al. 2021, Madenova et al. 2024]. Therefore, this result unearths a new question for further studies to test the relations among Niğde Misket Apple genotypes and the parental lineage of these cultivars. Although *Rvi6* is one of the most common *R* genes, studies indicated that the same origin of *V. inaequalis* populations has an infection on the *Rvi6* resistance allele carried and *M. floribunda* originated cultivars in Europe [Lemaire et al. 2016] and *Rvi6* resistance is suppressed and virulent progeny scatter, the plantation of *Rvi6* resistance allele-carrying cultivars is still a substantial factor for apple production to meet with sustainability requirements against apple scab [Peil et

al. 2018]. In terms of this aspect, Niğde Misket Apple may have an important advantage, and further studies related to the scab resistance can establish their basis for those results.

The results of QTL *FB_Mar12* can be an indication of the relationship between *M. floribunda*, *M. Evereste*, and *M.* × *arnoldiana* species to Niğde Misket Apple as well as the potential of the Niğde Misket Apple in terms of sustainable apple production against fire blight. But similar to the *Rvi6* results, additional research needs to be conducted in that manner for the results of QTL *FB_Mar12*. In terms of this aspect, Niğde Misket Apple may have an important advantage, and further studies related to the fire blight resistance can establish their basis for those results.

The presence of the S_{26} and S_9 alleles of the *S* gene shows the two different heritages of the Niğde Misket Apple genotypes. Because of the rare presence of the S_{26} allele, which is mostly reported on crabapples like Baskatong, *Malus floribunda* 821 [Broothaerts et al. 2004] and Marubakaido apple rootstock (*Malus prunifolia* Borkh.) [Brancher et al. 2020], Niğde Misket Apple probably had some relation to crabapples in its heritage. However, given the limited scope of this study, this hypothesis requires further investigation. Also, some of the apple genotypes from Türkiye have the presence of the S_{26} allele [Karataş et al. 2023], Niğde Misket Apple genotypes have this fundamental basis of Türkiye apple genotypes. Although Niğde Misket Apple genotypes share the same basis with crabapples in terms of the S_{26} allele, they also have the presence of the S_9 allele. From this point of view, they also have a relation to the parental lineage of known apple cultivars like Starking Delicious, Fuji, and Jonagold [Broothaerts et al. 2004], in terms of the S_9 allele. For future breeding efforts, those results can be utilized for self-incompatibility aspects.

CONCLUSION

This study evaluated Niğde Misket Apple genotypes regarding fire blight and apple scab resistance as well as self-incompatibility with some known molecular markers. Results provided genotypes that had resistance alleles of *Rvi6* and QTL *FB_Mar12* with respective molecular markers. Those results can be the basis for further scab and fire blight resistance-related research to enhance and test in line with the results. Genetic diversity of genotypes has already been indicated in previous studies; no further analysis has been conducted in that manner. From a self-incompatibility point of view, genotypes have relations to crabapple as well as the parental lineage of known apple cultivars for a specific allele of the *S* gene. These relationships should be further investigated to determine whether they are limited to *S* genes or involve additional genetic similarities that could be leveraged in plant breeding. New hypotheses emerged from this study. With future studies, more interpretation for the potential of the Niğde Misket Apple for plant breeding can be revealed.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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