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Detecting Locus of Resistance to Scab in Local Apple Varieties in Uzbekistan Using Molecular Genetic Markers

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Abstract: The apple tree (*Malus* sp.) is the most cultivated plant in the world. Apple orchards cover a large area. scab (*Venturia inaequalis*) causes significant damage to the economies of many countries that are engaged in the production of apple trees. In Uzbekistan, most of the orchards are apple orchards. The present study includes a molecular genetic analysis of the resistance of local apple cultivars to pathogens *Venturia inaequalis* in local apple cultivars grown in Uzbekistan and the results of identification of resistance genes. The paper presents the results of molecular genetic analysis of the combination of scab resistance genes (Vf, Vfa1, Vfa2) found in 15% of samples. In 58.7% of the studied samples, a “high level of resistance” to scab was revealed.

Keywords: Polymorphism, Genetic markers, Phytopathogens, *V. inaequalis*

Introduction

The subfamily Maloideae to which the apple tree belongs includes 22-25 genera and about 600 species, which grow mainly in the temperate zone of the northern hemisphere. The genus *Malus*, belonging to this subfamily, is the most important in terms of the national economy. This genus has a wide distribution in various eco-geographical conditions. The range of the genus is within Europe, Asia and North America. The center of origin of the domestic apple tree is the Central Asian Genetic Center (Pereira-Lorenzo et al., 2009).

Eleven years ago, the nucleotide sequence of the apple tree was published (Tartarini et al., 1999), which opened up prospects for further study of the apple genome. The use of molecular genetic technologies, in particular, sequencing of the apple tree genome, revealed that the ancestor of *Malus domestica* is the wild species *Malus sieversii* (Velasco Riccardo et al., 2010).

SSR analysis has already been successfully applied to the apple tree to identify intraspecific polymorphism, determine varietal affiliation, and build a genetic map of the species *Malus domestica* (Gianfranceschi et al., 1996; Guilford P et al., 1997; Hokanson et al., 1998).

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At present, a large number of microsatellite (SSR) apple markers are known and even their database, GDR (Genome Database for Rosaceae), has been created, which provides information on markers of many Rosaceae, including apple. Information about the apple markers themselves is available at <https://www.rosaceae.org/search/markers>. The first large group of SSR markers, in the amount of 140 pieces, which served as the basis for the database and further research, was developed by a group of European researchers (Liebhard et al., 2002). Scab is a widespread disease of the apple tree, causes a decrease in the quality of fruits, and in the years of epiphytosis is the cause of mass abscission of ovaries and premature leaf fall. Affecting seedlings, causes them to stunt growth and reduce the quality of planting material. Different varieties of apple trees are affected by scab to varying degrees

Currently, at least 20 scab resistance genes are known. The first 8 of them: Va, Vb, Vbj, Vg, Vf, Vh, Vm and Vr are well studied and identified over 15 years ago. At present, the apple genome has markers linked to scab resistance genes Vf, Vh2, Vh4, and Vbj (Gessler et al., 2006). Later, several more genes were identified, and a new nomenclature was proposed.

Method

Sample Collection and DNA Isolation

109 samples were selected from about 70 varieties from the Surkhandarya, Khorezm regions of Uzbekistan and the Republic of Karakalpakstan.

Selection of Molecular Markers

Based on the analysis of the literature, markers identifying genes for resistance to apple scab, bacterial blight, and powdery rose were studied. STS marker VfC . Afunyan M.R. regularly belongs to the Vfa1, Vfa2, Vfa4 genes in the scab resistance locus Vf, the codominant Al-07, the dominant marker AM-19, disseminated by Tartarini et al., are specific to the Vf gene (Rvi6). There are also multiplex scab resistance kits using fluorescent markers CH-Vf (Vf), CH01d03 (Vg), CH02c02a (Vr2), Hi07h02 (Vm).

Table1. Molecular markers used to identify resistance genes

Disease	Marker	Gene	Primers	Product Size
Scab	VfC	Vfa1, Vfa2, Vfa4	F: 5`-ggttccaaagtccaattcc-3` R: 5`-cgtagcattttgagttgac-3`	286, 484, 646 bp
	AL07	Vf, vf	F: 5`-tggaagagagatccagaaagtg-3` R: 5`-catccctccacaaatgcc-3`;	466, 724 bp
	AM19	Vf	F: 5`-cgtagaacggaattgacagtg-3` R: 5`-gacaaaggcttaagtgtctcc-3`	526 bp

PCR Process

The PCR process for detection of resistance genes was carried out using a lyophilized ready-made PCR core. The total volume of the mixture of reaction products was 20 µl, including 1 µl of forward and reverse primers (0.1–0.5 µM), 1 µl of gDNA (≥20 ng) was added. The PCR amplification process was carried out in a Thermocycler VERITI amplifier according to the following program: initial denaturation at 94 °C for 5 minutes, then 35 cycles: 94 °C – 25 seconds, 56 °C – 30 seconds, 72 °C – 35 seconds , the final elongation was carried out at 72°C for 5 minutes. The amplification products were separated by gel electrophoresis on a 2.5% agarose gel. 100 bp DNA marker was used to determine the length of the amplicons.

Results and Discussion

The study included 109 local varieties of apple trees from different regions of Uzbekistan – Surkhandarya, Khorezm regions and Karakalpakstan, which were assessed for the presence of Vf genes associated with resistance to scab (*Venturia inaequalis*) using DNA markers.

The VfC 1/2 marker was used to identify the Rvi6 scab resistant gene in local apple varieties. PCR products should generate 286, 484, 646 b.p. fragments on the electrophorogram. Fragments 484, 646 bp produced by the VfC 1/2 marker belong to both resistant and susceptible genotypes of apple varieties to powdery mildew, and apple genotypes with a fragment of 286 bp indicate the presence of the powdery mildew resistance gene Rvi6 (Fig. 1).

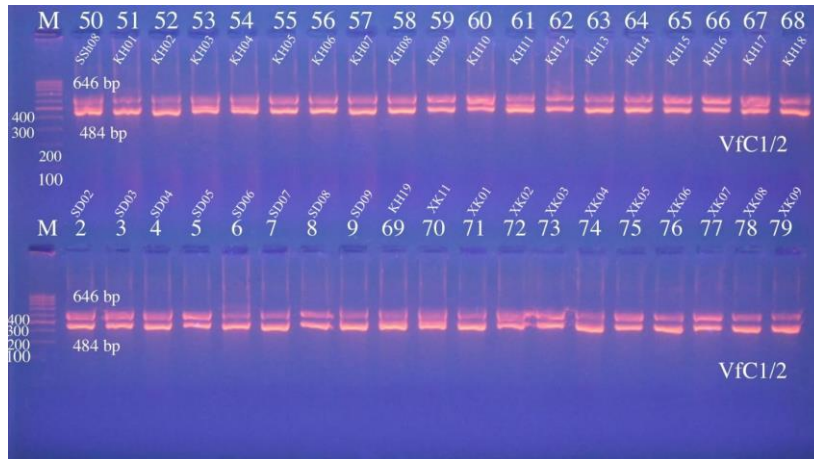


Figure 1. Amplicon fragments of the VfC1/2 marker. PCR product. M-marker.

The following distribution of allelic variants of genetic loci was revealed: Vfa1 (646 bp) was detected in 39 samples, Vfa2 (484 bp) in 106 samples, Vfa4 (286 bp) was not detected in any sample, AM19 Vf (526 bp) in 39, AL07 Vf (466 bp) was not detected in any sample, AL07 vf (724 bp) was present in all 109 samples (Fig. 2).

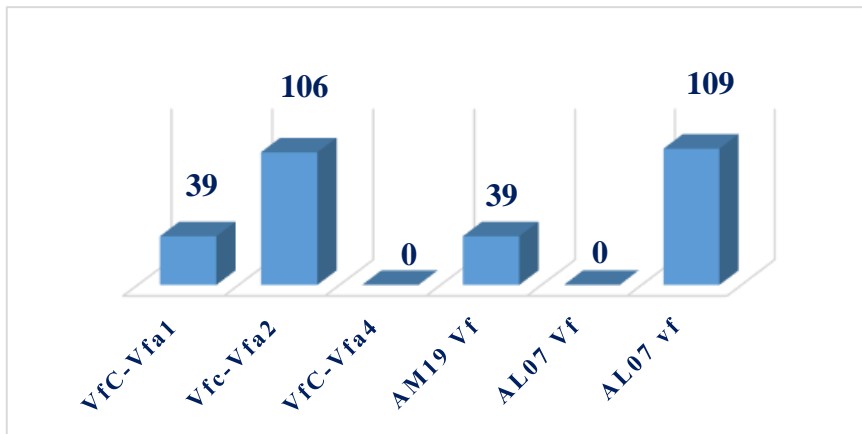


Figure 2. Distribution of occurrence of allelic variants of scab resistance genes for the three markers used

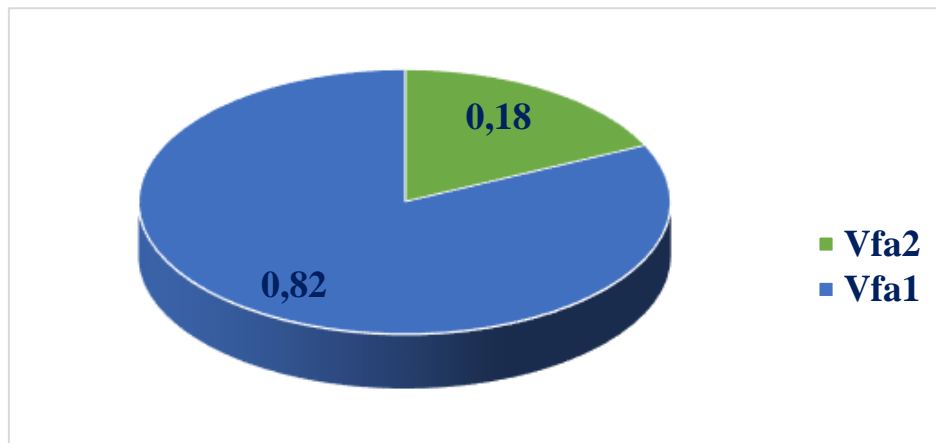


Figure 3. Distribution of Vfa1 and Vfa2 genotypes by VfC marker

Analysis of the distribution of genotypes revealed the presence of 2 homozygous genotypes for Vfa1, 72 homozygotes for the Vfa2 gene, and heterozygous Vfa1/Vfa2 genotypes were detected in 35 (32.1%) samples. The frequency distribution of Vf variants of the scab resistance gene in the apple population was presented as follows: Vfa1 - 0.18, Vfa2 - 0.82 ($\chi^2=0.94$; $p=0.33$). Thus, the frequency distribution of the three genotypes is in accordance with the equilibrium distribution of genotypes according to the Hardy-Weinberg law. A graphical representation of the frequency distribution of genotypes and alleles is shown in Fig. 3 and Fig. 4, respectively.

At least 10 scab resistance genes Vg, Vh2, Vh4, Vm, Vf, Va, Vbj, Vb, Vd and Vr2 are known (Ozgonen et al., 2007; Maric et al., 2010). For simplicity, Bus et al. (2009) proposed a new nomenclature for the above genes Rvi1, Rvi2, Rvi4, Rvi5, Rvi6, Rvi10, Rvi11, Rvi12, Rvi13 and Rvi15, respectively. In this work, we used the old nomenclature.

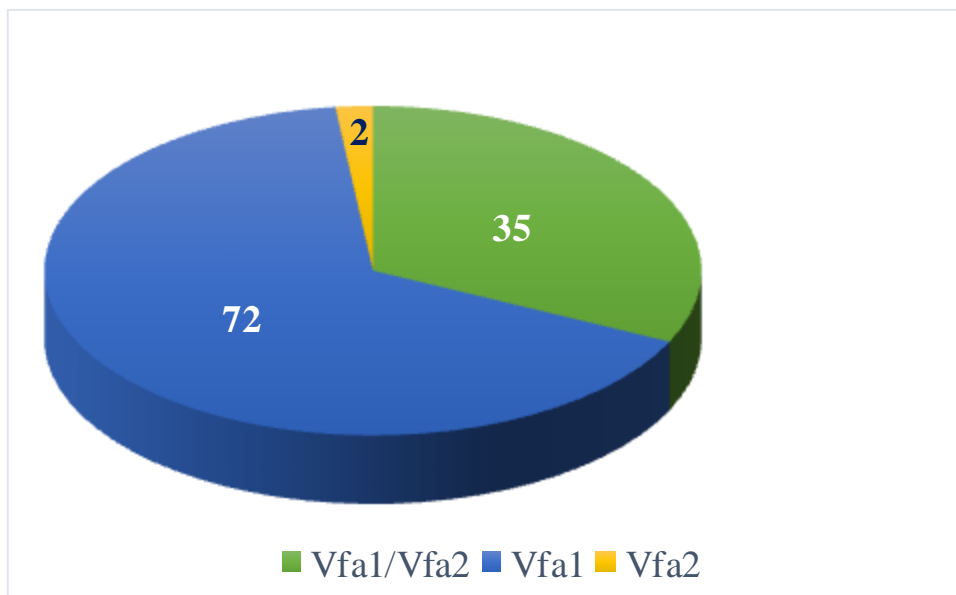


Figure 4. Frequency distribution of Vfa1 and Vfa2 variants by VfC marker

Of the 10 genes listed above, we decided to start with the genotyping of apple cultivars for the Vf genes, since this locus is the best studied and was one of the first identified in *Malus floribunda*, and this apple species has become the most widely used in breeding for the transfer of the resistance gene to commercial apple cultivars. scab.

In our study using the VfC marker, the Vfa1 gene was detected in 39 samples, Vfa2 in 106, and in 35 samples the presence of both (heterozygous) variants (Vfa1/Vfa2) was detected, while Vfa4 was not detected in any sample. It should be noted that in only two varieties of *M.domestica*, namely, "Besh barmok" (XS01) and "Kandolma" (XS13), the Vfa1 gene was detected in the homozygous state. Thus, according to the VfC marker, all the studied samples are to some extent resistant to scab (races 1 to 5).

According to previous studies, the use of the AL07 marker makes it possible to identify two variants of the Vf and vf gene (Tartarini et al., 1999; Patrascu et al., 2006), and the Vf variant found in *Malus floribunda* is dominant (manifested even in the heterozygous - Vf vf state) and is responsible for resistance to scab, while the vf variant is not associated with resistance.

In all analyzed samples for the AL07 marker, the Vf variant associated with resistance was not detected. Thus, according to the AL07 marker, all the studied samples are to some extent resistant to scab. Both markers - VfC and AL07 are codominant, i.e. they allow to detect the presence of at least two variants, while the AM19 Vf marker is a dominant marker, i.e. only one variant of the target Vf locus (yes/no) is detected during the analysis (Patrascu et al., 2006).

According to previous studies on a large sample of plants, the AM19 marker is much closer to the Vf genes than AL07; therefore, its linkage to the target resistance locus is less susceptible to recombination. According to the results of our study, the dominant marker AM19 made it possible to identify the Vf locus in 39 apple varieties.

Thus, the scab resistance gene for the AM19 marker was detected in 39 accessions, while the absence of this locus was found in 70 apple varieties.

Based on the obtained data of molecular genetic identification of Vf genes using three DNA markers, a table of the degree of resistance of each variety was compiled. The degree of resistance to each variety was assigned conditionally depending on the presence or absence of a locus of resistance or instability. Since the resistance locus Vf (AL07) was not detected in any sample in our study, its total contribution, as well as its recessive variant (vf), was not taken into account.

A value of 0 corresponds to the complete absence of marker loci associated with scab resistance genes (not detected in our study). A value of 1 was assigned if only the Vfa1 locus was present. A value of 2 was assigned if Vfa1 and Vfa2, or Vfa1 and Vf (AM19) loci were present. A value of 3 was assigned if only the Vfa2 locus or Vfa1, Vfa2 and Vf (AM19) was present. A value of 4 was assigned if both Vfa2 and Vf (AM19) loci were present in the variety. Analysis of the data showed that the main number (58.7%) of the samples has a 3 degree of stability, followed by samples with 2 and 4 degrees of stability 19.3% and 20.2%, respectively (Table 2).

Table 2. Distribution of varieties according to the degree of resistance to scab based on the genotype.

Degree of stability	Number of samples	Genotype (combinations of loci)
0	0	-
1	2	<i>Vfa1</i>
2	21	<i>Vfa1 + Vfa2</i> , либо <i>Vfa1 + Vf (AM19)</i>
3	64	<i>Vfa2</i> , либо <i>Vfa1 + Vfa2 + Vf (AM19)</i>
4	22	<i>Vfa2 + Vf (AM19)</i>

The absence of the *M.floribunda* 821 locus in some resistant apple varieties presented in Uzbekistan indicates that these varieties may have been obtained without the participation of the genetic material of *M. floribunda* 821, or another locus, which provides resistance, was transferred to some races of the pathogen.

Conclusion

Among the studied samples of the apple population, the Vf and Vfa1 genes were found in 35.8% of the samples, and the Vfa2 gene in 95.4%. The distribution combination of resistance genes showed that the Vfa2 and Vfa1 gene was found in 18 samples (16%), the Vfa2 and Vf gene in 22 samples (20%), Vfa1 and Vf only in 1 sample, the Vfa2, Vfa1 and Vf genes were found in 16 samples (15%), Vfa2 and vf genes in 48 samples (44%).

Thus, it was revealed that among the local varieties of apple trees, Maisky and Yanar showed high resistance to scab in terms of molecular genetic indicators, and the varieties Atlas olma, Turkish, Khuboni, Renat Semerenko, Shoyi olma, Besh bermok showed resistance to scab. Of the 109 samples of local varieties of apple trees studied, molecular genetic analyzes revealed high resistance to scab in 19.6% of the samples.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Acknowledgements or Notes

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