



DOI: 10.22363/2312-797X-2025-20-3-344-353

EDN USWZLV

UDC 634:11:631.52

Research article / Научная статья

Marker selection of promising varietal and breeding material of apple tree

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Abstract. Apple tree (*Malus × domestica* Borkh.) is a leading agricultural pome crop, one of the main perennial fruit plants in the world, commercially in demand and widespread in the horticultural industry of Russia, including the North Caucasus region. In solving the problem of creating high-quality domestic apple varieties with a long-term type of resistance to the scab (pathogen — *Venturia inaequalis* (Cooke) G. Winter), the role of prebreeding, including research aimed at accelerated selection using the DNA method, is significant tagging valuable carriers of several *Rvi* scab resistance genes. The purpose of the study was to study the allelic polymorphism of 15 apple tree scab resistance genes (*Venturia inaequalis* (Cooke) G. Winter) in 33 varietal and breeding samples of apple trees to identify sources of long-term resistance to increase efficiency of breeding process. The research was carried out in accordance with programs and methods for the study of apple varieties; Research and Selection Collection of Genetic Resources of Horticultural Crops and the Instrumentation and Analytical Center were used. DNA analysis methods were used in this work. For DNA extraction, a modification of the CTAB method was used, developed at North Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking, allowing for better purification of DNA samples from polyphenolic compounds. The work used 22 markers to identify 15 apple scab resistance genes: *Rvi1*; *Rvi2*; *Rvi3*; *Rvi4*; *Rvi5*; *Rvi6*; *Rvi8*; *Rvi9*; *Rvi11*; *Rvi12*; *Rvi13*; *Rvi14*; *Rvi15*; *Rvi16*; *Rvi17*. Marker selection of new varietal and breeding material made it possible to identify carriers of several genes for apple tree resistance to scab, having in the genome from 2 to 6 different *Rvi* genes in different combinations. Carriers of 6 resistance genes were identified as the most promising for selection for long-term resistance to scab: *Rvi1*, *Rvi2*, *Rvi3*, *Rvi4*, *Rvi6*, *Rvi15*–12/1–21–24; 5 genes: *Rvi1*, *Rvi2*, *Rvi3*, *Rvi6*, *Rvi13* — Karmen; *Rvi1*, *Rvi2*, *Rvi4*, *Rvi6*, *Rvi15* — Mikhsan; 4 genes: *Rvi1*, *Rvi2*, *Rvi3*, *Rvi6* — Nadezhnoye; *Rvi1*, *Rvi4*, *Rvi6*, *Rvi15* — Gaito Gazdanov; *Rvi1*, *Rvi3*, *Rvi6*, *Rvi8*–12/1–20–56, etc. Identified apple genotypes of domestic breeding, including those created in creative collaboration with colleagues

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from the Russian Research Institute for Breeding Fruit Crops and Stavropol Experimental Horticulture Station, promising for further breeding and accelerated creation of new high-quality, adaptive varieties with long-term high potential for resistance to the main fungal pathogen of the crop.

Key words: cultivar, hybrid, *Malus*, gene, DNA analysis, scab resistance

Authors' contribution: Ulianovskaya E.V. — concept development, analysis and interpretation of results, scientific writing; Chernutskaya E.A., Bogdanovich T.V., Tokmakov S.V., Stepanov I.V. — data collection and processing, approval of the final version of the manuscript.

Conflicts of Interest. The authors declare no conflict of interest.

Article history: received 10 April 2024; accepted 9 June 2025.

For citation: Ulianovskaya EV, Chernutskaya EA, Bogdanovich TV, Tokmakov SV, Stepanov IV. Marker selection of promising varietal and breeding material of apple tree. *RUDN Journal of Agronomy and Animal Industries*. 2025;20(3):344–353. doi: 10.22363/2312-797X-2025-20-3-344-353 EDN: USWZLV

Introduction

The apple tree is a vital perennial agricultural crop, the main fruit plant in the world, and the most widely distributed in the horticultural sector of Russia, including the North Caucasus. It is commercially attractive and popular among consumers of fruit products. The main resource for increasing Russian production of high-quality products of this leading fruit crop is selective renewal of the apple tree assortment based on productive varieties that are highly resistant to abiotic and biotic stressors in the cultivation region [1]. At the current stage of breeding work, it is necessary to combine increased indicators of adaptability, productivity, and fruit quality in the resulting genotypes, contributing both to an increase in profitability and economic efficiency of industrial production, and to successful competition in consumer market of new varieties with existing foreign analogues [2–7]. Given the need to address existing issues of biologization, ecologization, and rational nature management in the Russian horticultural industry, development of scab-resistant apple varieties is promising. This will enable the production of highly marketable fruits with commercially significant characteristics while improving the overall ecological situation in orchards [5, 8, 9].

The current apple assortment in the North Caucasus is quite diverse and extensive. As of 2024, the State Register of Breeding Achievements of the Russian Federation Approved for Use (State Register) lists 506 apple varieties, while the State Register for the North Caucasus Region lists 165 varieties¹. However, the significant and diverse quantitative composition and high rate of renewal of the apple assortment do not fully address the fundamental need to create and cultivate varieties that combine high resistance to biotic and abiotic stressors in the growing region with improved commercial fruit characteristics.

The use of modern methods for evaluating varietal and hybrid material to study biodiversity and identify the gene pool composition of the *Malus* Mill. genus is the basis

¹ Reestr selektsionnykh dostizhenii [State Register of Breeding Achievements]. Available from: <https://gossortrf.ru/registry/> (Accessed 22nd March 2024).

for the most effective development of high-quality and adaptable Russian varieties for southern fruit growing.

Modern methods for studying the genetics of key breeding traits, including long-term pathogen resistance, especially when combined with improved fruit quality, significantly facilitate the solution of critical breeding challenges in developing adaptive, competitive Russian varieties and enable the rapid selection of promising donors and complex donors of target economic traits for further breeding.

DNA analysis methods facilitate acceleration and optimization of several key stages in breeding process for perennial fruit crops, particularly apples. With the development of DNA technologies, comprehensive genotyping and phenotyping of perennial plants, including apples, is becoming increasingly common. The process of combining and integrating long-term breeding data with genetic research data plays an important role in improving apple breeding methods through the increasingly active use of markers, expanding the genetic understanding of the most important crop traits and ensuring maximum accessibility and high information content of the obtained data for breeders [10]. Marker-assisted selection of sources of target genes and gene alleles allows for a significant reduction in duration, labor intensity, and energy consumption of apple breeding process [11]. The use of DNA marking is important both at the initial stage of breeding process — for studying and selecting the most valuable parental forms — and later — for identifying promising hybrid material, elite forms, and new varieties.

The aim of the study was to investigate the allelic polymorphism of 15 genes of apple scab resistance (*Venturia inaequalis* (Cooke) G. Winter) in 33 apple cultivar and breeding samples and to identify sources of long-term resistance to enhance the efficiency of the breeding process.

Materials and methods

The objects of the study were 33 apple genotypes with different ecological, geographical, and genetic origins.

The study was conducted at North Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking. The research was conducted at two shared use centers (SUCs) — the Instrumentation and Analysis Center and the Research and Selection Collection of Genetic Resources of Horticultural Crops, located in the North Caucasus Horticultural Region of the Russian Federation (Krasnodar, ‘Tsentralnoye’ farm). Samples for DNA analysis were collected from collection and selection apple tree plantings grown on M9 rootstock in a 4 × 1 m pattern, planted in 2015–2017.

The study utilized programs and methods for breeding and variety study of fruit crops (apple trees)² [12]; molecular genetic methods for selecting sources of long-term apple resistance to scab that are valuable for further breeding [13, 14], and a DNA

² Egorov EA. (ed.) *Programma Severo-Kavkazskogo tsentra po seleksii plodovykh, yagodnykh, tsvetochno-dekorativnykh kul'tur i vinograda na period do 2030 goda* [Program of the North Caucasus Center for Breeding Fruit, Berry, Flower and Ornamental Crops and Grapes for the Period up to 2030]. Krasnodar, 2013.

analysis method based on PCR followed by electrophoretic analysis of the products. A modification of the CTAB method, based on a 1% concentration of polyvinylpyrrolidone in the lysis buffer, performed at North Caucasian Federal Scientific Center of Horticulture, Viticulture, Winemaking [15], was used, which improved the purification of DNA samples from polyphenolic compounds.

A total of 22 markers of 15 scab resistance genes were used: *Rvi1* — CH01D03 (SSR marker); *Rvi2* — CH02b10 (SSR marker); *Rvi3* — Hi08e04 (SSR marker); *Rvi4* — CH02c02a, CH02f06 (SSR marker); *Rvi5* — Hi07h02, FMACH_Vm3 (SSR markers); *Rvi6* — CH-Vf1 (SSR marker), VfC1F+VfC2 (SCAR marker); *Rvi8* — OPL 19 (SCAR marker); *Rvi9* — CH03d01, CH05e03 (SSR marker); *Rvi11* — CH05e03; *Rvi12* — SSR23.17, SSR24.91 (SSR markers); *Rvi13* — CH04f03, CH02b07 (SSR markers); *Rvi14* — HB09 (SSR marker); *Rvi15* — CH02c02a, CH02f06 (SSR markers); *Rvi16* — NH030a (SSR marker); *Rvi17* — CH-Vf1 (SSR marker).

The following varieties, species, and hybrid forms, included in international set of differentiators for determining racial composition of scab pathogen, were used as positive controls for molecular genetic identification of the DNA marker alleles of the desired apple scab resistance genes: Golden Delicious — *Rvi1* gene; *Malus pumila* R12740–7A — *Rvi2* gene; Q71 (*Rvi3*); *Malus atrosanguinea* 840 (*Rvi5*); Priscilla (*Rvi6*); B45 (*Rvi8*); J34 (*Rvi9*); *M. baccata jackii* (*Rvi11*); Hansen's baccata (*Rvi12*), Durello di Forli — (*Rvi13*); Dulmener Rosenapfel — (*Rvi14*); GMAL2473 (*Rvi15*).

Results and discussion

To identify carriers of potential long-term scab resistance and expand the identified portion of the apple tree collection, 33 accessions of new varietal and breeding material were analyzed at the North Caucasian Federal Scientific Center of Apple Tree Breeding using DNA marking. A phenotypic assessment was performed for a range of key economic, biological, and adaptive traits (timing of fruiting, main developmental stages, tree vigor, crown compactness and fruiting pattern, resistance to scab, moniliosis, powdery mildew, phyllostictosis, resistance to spring frosts and drought, regularity of fruiting, productivity, fruit quality, etc.). Phenotyping enabled the identification of 33 apple accessions (13 hybrids, 6 elite forms, 11 Russian and 3 foreign varieties) combining the maximum number of valuable traits for DNA analysis. Trait subcollections were formed. The sample set studied for DNA analysis included new varieties and elite selections from the North Caucasian Federal Scientific Center of Horticulture, Viticulture, and Winemaking, including those developed jointly with colleagues from the Russian Research Institute for Breeding Fruit Crops and Stavropol Scientific Center of Horticulture (Table 1).

Using DNA analysis, 15 genes were identified in the selected apple accessions: *Rvi1-Rvi6*, *Rvi8*, *Rvi9*, *Rvi11-Rvi17*, which determine resistance to scab of *Venturia inaequalis* (Cooke) G. Winter.

It was established that a significant portion (42.4%) of the studied accession sample carried *Rvi6* scab resistance gene. *Rvi6* gene (previously known as the *Vf* gene) is widespread and has been frequently used in apple breeding worldwide since the early 20th century. A study of new hybrid and varietal material allowed us to identify the CH-Vf1 and VfC1F+VfC2 markers (linked to the *Rvi6* gene) in the genomes of 14 of 33 accessions (5 varieties and 9 elite and selected forms).

Table 1

Origin of new apple varietal and breeding material

Genotype name	Country, originator	Origin
17/1-6-2	Russia, NCFSCHVW	Karmen × Gemeni
17/1-6-32	Russia, NCFSCHVW	Champion × Modi
17/1-6-57	Russia, NCFSCHVW	Liberty × Renuartsiv
17/1-6-65	Russia, NCFSCHVW	12/1-21-63 (Golden Delicious (4x) × 2034 [F ₂ <i>M. floribunda</i> × Golden Delicious]) × Modi
17/1-6-74	Russia, NCFSCHVW	12/1-21-63 (Golden Delicious (4x) × 2034 [F ₂ <i>M. floribunda</i> × Golden Delicious]) × Modi
17/2-6-9	Russia, NCFSCHVW	12/1-21-24 (Idared × Balsgard 0247E) × Arksharm
17/2-6-2	Russia, NCFSCHVW	Champion × Modi
17/1-7-2	Russia, NCFSCHVW	12/1-21-24 (Idared × Balsgard 0247E) × Arksharm
17/1-7-3	Russia, NCFSCHVW	12/1-21-24 (Idared × Balsgard 0247E) × Arksharm
17/1-7-16	Russia, NCFSCHVW	12/1-20-56 (Black Stayman × Prima) × Fujion
17/1-7-18	Russia, NCFSCHVW	12/1-20-56 (Black Stayman × Prima) × Fujion
17/1-7-26	Russia, NCFSCHVW	Champion × Modi
17/1-7-27	Russia, NCFSCHVW	Champion × Modi
12/1-20-56	Russia, NCFSCHVW, RRIFCB	Black Stayman × Prima
12/1-21-24	Russia, NCFSCHVW, RRIFCB	Idared × Balsgard 0247E
12/1-21-63	Russia, NCFSCHVW, RRIFCB	Golden Delicious (4x) × 2034 (F ₂ <i>M. floribunda</i> × Golden Delicious)
6-4-12	Russia, NCFSCHVW	44-30-7 (Welsey (4x) × Bessemyanka Michurinskaya) × Florina
6-6-11	Russia, NCFSCHVW	44-30-7 (Welsey (4x) × Bessemyanka Michurinskaya) × Florina
Egorovskoye	Russia, NCFSCHVW, ВНИИСПК, SSCH	Redfree × Papirova tetraploidnaya
Karmen	Russia, NCFSCHVW, RRIFCB	Prima × Welsey (4x)
Bagryanets Kubani	Russia, NCFSCHVW	Clone of cv. Kubanskoye Bagryanoye
Renet Platona	Russia, NCFSCHVW	Clone of cv. Renet Simirenko, mutation selection
Pamyati Yesaulu	Russia, NCFSCHVW	(Rosemarin × Prima) × Kandil Krasnodarsky
Prikubanskoye	Russia, NCFSCHVW	Red Delicious × Opalescent

Ending tabl. 1

Genotype name	Country, originator	Origin
Solnechnoye	Russia, NCFSCHVW	Clone of cv. Celeste, mutation selection
Delishes Marii	Russia, NCFSCHVW	Delicious spur × Kidd's Orange Red, mutation selection
Zimnee utro	Russia, NCFSCHVW	Liberty × Scarlett Staymared, mutation selection
Mikhsan	Russia, SSCH, NCFSCHVW	Liberty × Golden Delicious
Nadezhnoye	Russia, NCFSCHVW, RRIFCB, SSCH	Idared × Balsgard 0247E
Gaito Gazdanov	Russia, SSCH, NCFSCHVW, 'De-Gusto'	Golden Delicious × Liberty
Renuartsiv	Italy, C.I.V. – SOCIETA' CONSORTILE A R.L.	"Sweet Resistants" series, developed with cv. Granny Smith
Honey Crisp	USA, Minnesota Agricultural Experiment Station's Horticultural Research Center	Macoun × Honeygold
Red Delicious King Roat	Italy, KIKU GmbH	Clone of cv. Hapke

Source: compiled by E.V. Ulianovskaya, E.A. Chernutskaya.

Almost half of the samples carried *Rvi3* gene (16 of 33 studied, or 48.5%). The most common scab resistance genes in the sample also included *Rvi1*, *Rvi2*, and *Rvi8*, with frequencies in the sample of 42.4%, 33.3%, and 36.4%, respectively. The frequencies of *Rvi4*, *Rvi15*, *Rvi13*, and *Rvi14* in the sample were 24.2%, 18.2%, 9.1 %, and 9.1%, respectively.

The presence of four resistance genes was not detected: *Rvi5*, *Rvi11*, *Rvi12*, and *Rvi16*.

The frequencies of *Rvi17* and *Rvi9* genes were 6.1% and 3.0%, respectively, so these scab resistance genes can be considered relatively rare in the studied sample.

Carriers of several apple scab resistance genes have been identified, each with 2 to 6 different *Rvi* system genes in various combinations in their genome. These genes are valuable for further selection for long-term, persistent resistance to the pathogen (Table 2, Fig.)

Table 2

Carriers of apple scab resistance genes (*Rvi*)

Presence of scab resistance genes (<i>Rvi</i>)	Genotype name
<i>Rvi1</i> , <i>Rvi2</i> , <i>Rvi3</i> , <i>Rvi4</i> , <i>Rvi6</i> , <i>Rvi15</i>	12/1–21–24
<i>Rvi1</i> , <i>Rvi2</i> , <i>Rvi3</i> , <i>Rvi6</i> , <i>Rvi13</i>	Karmen
<i>Rvi1</i> , <i>Rvi2</i> , <i>Rvi4</i> , <i>Rvi6</i> , <i>Rvi15</i>	Mikhsan

Ending tabl. 2

Presence of scab resistance genes (<i>Rvi</i>)	Genotype name
<i>Rvi1</i> , <i>Rvi2</i> , <i>Rvi3</i> , <i>Rvi6</i>	Nadezhnoye
<i>Rvi1</i> , <i>Rvi4</i> , <i>Rvi6</i> , <i>Rvi15</i>	Gazdanov
<i>Rvi1</i> , <i>Rvi3</i> , <i>Rvi6</i> , <i>Rvi8</i>	12/1–20–56
<i>Rvi1</i> , <i>Rvi2</i> , <i>Rvi3</i> , <i>Rvi8</i>	17/1–6–57
<i>Rvi3</i> , <i>Rvi4</i> , <i>Rvi14</i> , <i>Rvi15</i>	17/1–7–3
<i>Rvi2</i> , <i>Rvi3</i> , <i>Rvi4</i> , <i>Rvi6</i>	17/1–7–16

Source: compiled by E.V. Ulianovskaya, E.A. Chernutskaya, T.V. Bogdanovich.



Karmen



Gaito Gazdanov



Mikhsan



Nadezhnoye

New Russian apple cultivars
Source: photo by E.V. Ulianovskaya.

It should be noted that the new breeding material was obtained by hybridization of promising varieties and forms of Russian, Western European, North

American and Japanese selection, possessing improved fruit quality indicators. A sufficiently high breeding value of the new breeding material is confirmed by a high proportion (84.2%, or 16 out of 19 studied accessions) of identified carriers of 2–4 scab resistance genes. It was established that a significant number of carriers of several scab resistance genes were created by geographically distant hybridization with the participation of elite forms of regional selection and Western European cultivars (12/1–21–63 and Modi), Japanese (12/1–20–56 and Fujion) or North American (12/1–21–24 and Arksharm); North American and Western European cultivars (Liberty and Renuartsiv). The results of marker-assisted selection of new breeding and varietal material for scab resistance genes are promising for expanding the identified composition of the apple gene pool and increasing the efficiency of further breeding.

Conclusion

According to the data obtained, carriers of several scab resistance genes were identified in the studied sample of 33 apple varieties and hybrid forms of different ecological, geographical, and genetic origins. These genes carried from 2 to 6 different *Rvi* genes in various combinations in their genomes. The most common scab resistance genes among the 15 studied genes were *Rvi3* and *Rvi6*, as well as *Rvi1*, *Rvi2*, and *Rvi8*. Carriers of the following scab resistance genes were identified: 6 scab resistance genes — 12/1-21-24; 5 resistance genes — Karmen and Mikhsan; 4 genes: Nadezhnoe, Gaito Gazdanov, 12/1-20-56, 17/1-6-57, 17/1-7-3, 17/1-7-16, valuable for breeding for long-term resistance to biopathogens.

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Маркерный отбор перспективного сортового и селекционного материала яблони

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Аннотация. Яблоня (*Malus × domestica* Borkh.) — ведущая сельскохозяйственная семечковая культура, одна из основных среди многолетних плодовых растений мира, коммерчески востребована и широко

распространена в отрасли садоводства России, в т. ч. региона Северного Кавказа. В решении проблемы создания высококачественных российских сортов яблони с долговременным типом устойчивости к парше (основному заболеванию культуры; возбудитель — *Venturia inaequalis* (Cooke) G. Winter) значительна роль предбридинга, в т. ч. исследований, направленных на ускоренный отбор методом ДНК-маркирования ценных носителей нескольких генов *Rvi* устойчивости к парше. Цель исследования — по результатам изучения аллельного полиморфизма 15 генов устойчивости яблони к парше (*Venturia inaequalis* (Cooke) G. Winter) у 33 сортовых и селекционных образцов яблони выделить источники долговременной устойчивости для усиления эффективности селекционного процесса. Исследование проводили согласно программам и методикам по сортоизучению яблони; использованы «Исследовательско-селекционная коллекция генетических ресурсов садовых культур» (ЦКП ИСК ГРСК) и ЦКП «Приборно-аналитический». Применен ДНК-маркерный анализ. Для экстракции ДНК применяли метод СТАВ в модификации Северо-Кавказского федерального научного центра садоводства, виноградарства, виноделия, что позволило вести более качественную очистку подготовленных проб ДНК от полифенольных соединений. Использованы 22 маркера для идентификации 15 генов устойчивости яблони к парше: *Rvi1*; *Rvi2*; *Rvi3*; *Rvi4*; *Rvi5*; *Rvi6*; *Rvi8*; *Rvi9*; *Rvi11*; *Rvi12*; *Rvi13*; *Rvi14*; *Rvi15*; *Rvi16*; *Rvi17*. Маркерный отбор нового сортового и селекционного материала позволил выделить носителей нескольких генов устойчивости яблони к парше, имеющих в геноме от 2 до 6 различных генов *Rvi* в разных вариантах сочетания, это носители 6 генов: *Rvi1*, *Rvi2*, *Rvi3*, *Rvi4*, *Rvi6*, *Rvi15* — 12/1–21–24; 5 генов в сочетаниях: *Rvi1*, *Rvi2*, *Rvi3*, *Rvi6*, *Rvi13* — Кармен; *Rvi1*, *Rvi2*, *Rvi4*, *Rvi6*, *Rvi15* — Михсан; 4 генов в сочетаниях: *Rvi1*, *Rvi2*, *Rvi3*, *Rvi6* — Надежное; *Rvi1*, *Rvi4*, *Rvi6*, *Rvi15* — Гайто Газданов; *Rvi1*, *Rvi3*, *Rvi6*, *Rvi8*–12/1–20–56 и др. Выделенные генотипы яблони российской селекции, в т. ч. созданные в творческом содружестве с коллегами из Всероссийского НИИ селекции плодовых культур (ВНИИСПК) и Ставропольской ОС садоводства (СОСС), перспективны для дальнейшей селекции и ускоренного создания новых качественных, адаптивных сортов с длительным высоким потенциалом устойчивости к основному грибному патогену культуры.

Ключевые слова: сорт, гибрид, *Malus*, ген, ДНК-анализ, устойчивость к парше

Вклад авторов: Ульяновская Е.В. — разработка концепции, анализ и интерпретация данных, подготовка и редактирование текста; Чернуцкая Е.А., Богданович Т.В., Токмаков С.В., Степанов И.В. — сбор и обработка материалов, утверждение окончательного варианта статьи.

Заявление о конфликте интересов. Авторы заявляют об отсутствии конфликта интересов.

История статьи: поступила в редакцию 10 апреля 2024 г., принята к публикации 9 июня 2025 г.

Для цитирования: Ульяновская Е.В., Чернуцкая Е.А., Богданович Т.В., Токмаков С.В., Степанов И.В. Маркерный отбор перспективного сортового и селекционного материала яблони // Вестник Российского университета дружбы народов. Серия: Агрономия и животноводство. 2025. Т. 20. № 3. С. 344–353. doi: 10.22363/2312-797X-2025-20-3-344-353 EDN: USWZLV