Assessment of strawberry varieties by anthracnose resistance gene

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Abstract. The results of marker-assisted identification of the *Rca2* anthracnose resistance gene in promising strawberry varieties were shown. The DNA marker STS-Rca2_240, linked to the *Rca2* anthracnose resistance allele was identified in varieties Albion and Aprica (*F.* × *ananassa*), and variety Dar (*F.* × *anashata*). Strawberry varieties Alpha, Bereginya, Kokinskaya Zarya, Pamyati Zubova, Rubinovy kaskad, Slavutich, Yuniol, Big King, Brilla, Joly, Rumba and Vivara are characterized by the absence of the marker STS-Rca2_240. In strawberry varieties with the identified marker STS-Rca2_240 (Dar, Albion and Aprica), the *Rca2* gene can be in a dominant homozygous or heterozygous state, which makes it possible to recommend these genotypes for use in breeding for anthracnose resistance.

Strawberry is susceptible to many fungal, bacterial and viral diseases. The most important strawberry fungal diseases are powdery mildew, gray mold rot, white and brown leaf spots, anthracnose, red stele root rot and verticillium wilt [1-4].

Anthracnose is one of the most dangerous fungal diseases of strawberry. Anthracnose is caused by phytopathogenic fungi of the genus *Colletotrichum*: *Colletotrichum acutatum* J.H. Simmonds, *C. gloeosporioides* (Penz.) Penz. & Sacc. And *C. fragariae* A.N. Brooks (some researchers combine *C. fragariae* and *C. gloeosporioides* into the *C. gloeosporioides* species complex) [5-7]. In the temperate climatic zone, the *C. acutatum* species complex is most common [8-10]. In Europe and the Eurasian Economic Union, *C. acutatum* is a quarantine pathogen [11, 12].

According to the studies of European researchers, the strawberry resistance to some isolates of *C. acutatum* is controlled by the dominant *Rca2* gene. Identification of genetic determinants of this trait allows for targeted selection of promising strawberry forms using diagnostic molecular markers [13-15].

The purpose of the study was assess the allelic diversity of the *Rca2* anthracnose resistance gene in strawberry varieties to identify promising genotypes for involvement in the strawberry breeding to create resistant to fungal pathogens forms.

The studies were carried out in 2020-2021. Biological material was represented by promising strawberry varieties from genetic collection of the FSSI "I.V. Michurin Federal Scientific Center" (Table 1).

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Table 1. Analyzed strawberry varieties

Variety	Crossing combination	Origin / Originator
Dar	*F.* × *ananassa* Duch. × *F. moschata* Duch.	Russia
Alpha	Festivalnaya romashka × Syurpriz olimpiade	Federal Horticultural Research Center for Breeding, Agrotechnology and Nursery, Russia
Bereginya	Solovushka × Induka	
Kokinskaya zarya	Slavutich × 157-7	
Slavutich	Festivalnaya romashka × Syurpriz olimpiade	
Pamyati Zubova	[Feyerverk × (Belrubi × *F. ovalis* Rydb.)] × Holiday	I.V. Michurin Federal Scientific Center, Russia
Rubinovyy kaskad	922-67 × Privlekatelnaya	
Yuniol	No information available	The Labor Red Banner Order Nikita Botanical Gardens – National Scientific Center of Russian Academy of Sciences, Republic of Crimea, Russia
Big King	No information available	Russia
Albion	Diamante × Cal 94.16-1	University of California Davis, USA
Brilla	FC 04.256.32	CRA-Unità di Ricerca per la Frutticoltura, Italy
Joly	T2-6 × A20-17	Consorzio Italiano Vivaisti (CIV), Italy
Aprica	No information available	
Laetitia (control)	No information available	
Vivara	No information available	
Rumba	No information available	Fresh Forward B.V., Netherlands

The allelic state of the *Rca2* anthracnose resistance gene was identified by DNA analysis using the diagnostic marker STS-Rca2_240. The marker STS-Rca2_240 is represented by a 240 bp amplicon, which is amplified only if the *Rca2* anthracnose resistance allele is present in the strawberry genotype. The SSR marker EMFv020 was used as the positive PCR control. The target fragment of marker EMFv020 (amplicon size about 170 bp) is present in all strawberry genotypes [16]. The control of the presence of the *Rca2* anthracnose resistance allele in the genome was the strawberry variety Laetitia. According to previous studies, the strawberry variety Laetitia is characterized by a dominant homozygous or heterozygous genotype (*Rca2Rca2* or *Rca2rca2*) [17].

Polymerase chain reaction (PCR) was performed in T100 Thermal Cycler (BIO-RAD) according to the previously described program [17, 18].

Amplification products were separated by electrophoretic method in agarose gel (agarose concentration – 2%, running buffer – 1x TBE). Amplicon sizes estimated were performed using the Gene Ruler 100 bp DNA Ladder (Thermo Fisher Scientific).

In the analyzed strawberry collection, the marker STS-Rca2_240 was identified in the varieties Albion and Aprica (*F.* × *ananassa*), and variety Dar (*F.* × *anashata*). Strawberry varieties Alpha, Bereginya, Kokinskaya Zarya, Pamyati Zubova, Rubinovy kaskad, Slavutich, Yuniol, Big King, Brilla, Joly, Rumba and Vivara are characterized by the absence of the target fragment of the STS-Rca2_240 marker. The example of

electrophoretic profiles of marker fragments of the *Rca2* gene in strawberry varieties is shown in Figure; the results are shown in Table 2.

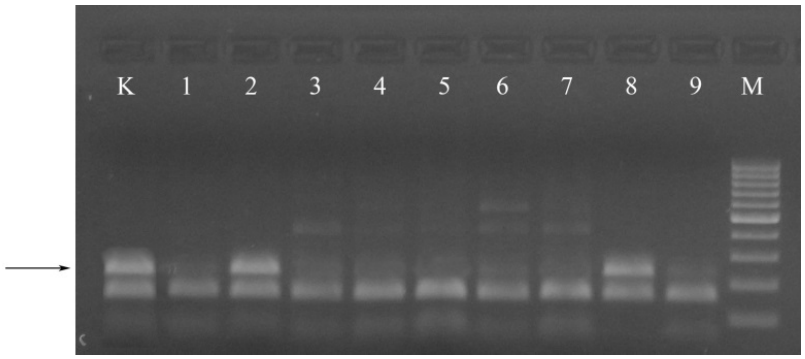

Fig. Electrophoresis profile of marker STS-Rca2_240 at strawberry varieties
K – control (Laetitia), 1 – Rumba, 2 – Aprica, 3 – Pamyati Zubova, 4 – Rubinovyy kaskad, 5 – Brilla, 6 – Big king, 7 – Slavutich, 8 – Albion, 9 – Yuniol, M – Molecular weight marker

Table 2. Polymorphism of the *Rca2* anthracnose resistance gene in strawberry varieties (1 – allele is present, 0 – allele is absent)

№	Variety	Marker STS-Rca2_240	Genotype
1	Alpha	0	*rca2rca2*
2	Bereginya	0	*rca2rca2*
3	Dar	1	*Rca2Rca2* or *Rca2rca2*
4	Kokinskaya zarya	0	*rca2rca2*
5	Pamyati Zubova	0	*rca2rca2*
6	Rubinovyy kaskad	0	*rca2rca2*
7	Slavutich	0	*rca2rca2*
8	Yuniol	0	*rca2rca2*
9	Albion	1	*Rca2Rca2* or *Rca2rca2*
10	Aprica	1	*Rca2Rca2* or *Rca2rca2*
11	Big King	0	*rca2rca2*
12	Brilla	0	*rca2rca2*
13	Joly	0	*rca2rca2*
14	Laetitia (control)	1	*Rca2Rca2* or *Rca2rca2*
15	Rumba	0	*rca2rca2*
16	Vivara	0	*rca2rca2*

It should be noted that strawberry varieties Pamyati Zubova, Big King and Slavutich, in addition to fragments of a known size, have additional amplicons. Strawberry varieties Pamyati Zubova and Slavutich have an amplicon of about 400 bp. Strawberry variety Big king has an amplicons of about 400 and 500 bp. These amplicons are presumably unrelated to anthracnose resistance.

Strawberry varieties with the identified marker STS-Rca2_240 (Dar, Albion and Aprica) are characterized by the presence of the *Rca2* anthracnose resistance allele in a dominant homozygous or heterozygous state. To accurately determine the allelic state of the *Rca2* gene in these strawberry varieties, additional research is needed.

Thus, as a result of the molecular genetic analysis, the *Rca2* anthracnose resistance gene was identified in varieties Albion and Aprica (*F.* × *ananassa*), and variety Dar (*F.* ×

anashata), which makes it possible to recommend these genotypes for use in breeding to create resistant to *C. acutatum* strawberry varieties.

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