Analysis of strawberry promising varieties and selected forms by resistance to red stele root rot using molecular markers

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Abstract. The analysis of the allelic state of the *Rpf1* red stele root rot gene in 14 promising foreign strawberry varieties and 6 selected forms of the I.V. Michurin FSC breeding was completed. The *Rpf1* gene in a heterozygous state was identified in strawberry forms 61-15 (Bylinnaya × Olimpiyskaya nadezhda), 69-29 (Feyerverk × Bylinnaya), and 72-71 (Privlekatelnaya × Bylinnaya), which makes it possible to recommend them for involvement in breeding work to create resistant to *P. fragariae* var. *fragariae* strawberry varieties. Strawberry varieties Lebedushka, Elianny, Florence, Malwina, Monterey, Polka, Verona, Vima Tarda Asia, Chamora Turusi, Clery, Flamenco, Salsa and Symphony, and selected forms 56-5 (Gigantella Maxim × Privlekatelnaya), 69-42 (Feyerverk × Bylinnaya) and 35-16 (922-67 × Maryshka) have a recessive homozygous genotype.

1 Introduction

Resistance to fungal pathogens is one of the most important traits of any plant variety. Strawberry is susceptible to many diseases that affect all parts of plants: leaves, roots, flowers, fruits, etc. One of the most dangerous diseases of strawberry is diseases of the root system. Among them is red stele root rot, the causative agent of which is the quarantine phytopathogen *Phytophthora fragariae* var. *fragariae* Hickman [1-3]. In strawberry plants affected by red stele root rot, the death of lateral feeding and adventitious roots is observed with the formation of pathogen oospores in the affected tissues, inhibition of growth and wilting [4, 5].

Strawberry resistance to red stele root rot is due to the presence in the genome of several race-specific genes, of which the *Rpf1*, *Rpf2*, and *Rpf3* genes are the most important [6, 7].

The purpose of this study was the molecular genetic analysis of the allelic state of the *Rpf1* gene in promising strawberry varieties and selected forms for identification of resistant to *P. fragariae* var. *fragariae* genotypes.

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2 Materials and methods

The studies were carried out in 2020-2021. Biological material was represented by widespread industrial strawberry varieties, introduced from various ecological and geographical regions of growth and promising strawberry hybrid seedlings obtained at the "I.V. Michurin Federal Scientific Center" (Table 1).

Table 1. Analyzed strawberry varieties and selected forms

Variety / form	Crossing combination	Originator
Lebedushka	No information available	Ukraine
Asia	NF421	New Fruits, Italy
Chamora Turusi	No information available	Japan
Clery	Sweet Charlie × Onebor	Consorzio Italiano Vivaisti, Italy
Elianny	No information available	Gebr. Vissers, Netherlands
Flamenco	Evita × EMR77	East Malling Research Station, United Kingdom
Florence	[Tioga × (Red Gauntlet × (Wiltguard × Gorella))] × (Providence × self)	MEIOSIS LTD, United Kingdom
Malwina	Sophie × clone Schimmelpfeng, Weihenstefan	Peter Stoppel, Germany
Monterey	Cal. 27−85.06 × Albion	University of California Davis, USA
Polka	Unduka × Sivetta	Plant Research International - WUR, Netherlands
Salsa	No information available	Fresh Forward B.V., Netherlands
Symphony	Rhapsody × Holiday	Mylnefield Research Services Ltd, United Kingdom
Verona	No information available	Fragolà, Italy
Vima Tarda	Vima Zanta × Vicoda	Vissers International BV, Netherlands
35-16	922-67 × Maryshka	"I.V. Michurin Federal Scientific Center", Russia
56-5	Gigantella Maxim × Privlekatelnaya	
61-15	Bylinnaya × Olimpiyskaya nadezhda	
69-29	Feyerverk × Bylinnaya	
69-42		
72-71	Privlekatelnaya × Bylinnaya	

The allelic state of the *Rpf1* gene was identified by DNA analysis using diagnostic markers OPO-16C (RAPD) [8] and R1A (SCAR) [9].

The RAPD marker OPO-16C is represented by amplicons from 400 to 2000 bp. The 438 bp amplicon is linked to the *Rpf1* gene (non-functional *rpf1* allele). Other products of the OPO-16C marker are amplified regardless of the allelic state of the *Rpf1* gene [8, 10]. The SCAR marker R1A is represented by a 285 bp amplicon and linked to the functional allele *Rpf1* [9]. The use of a combination of markers OPO-16C and R1A makes it possible to identify the allelic state of the *Rpf1* gene: the presence of the R1A marker and the absence of the OPO-16C marker indicates a dominant homozygous genotype, the presence of both markers indicates a heterozygous genotype, the presence of the OPO-16C marker and the absence of the R1A marker, and the absence of both markers, indicates a recessive homozygous genotype [11].

Polymerase chain reaction (PCR) was performed in T100 Thermal Cycler (BioRad) according to the previously described programs [10, 12].

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).

3 Results and discussion

According to the research, the OPO-16C marker was identified in the strawberry varieties Lebedushka, Elianny, Florence, Malwina, Monterey, Polka, Verona and Vima Tarda, and selected strawberry hybrid forms 56-5 (Gigantella Maxim × Privlekatelnaya), 61-15 (Bylinnaya × Olimpiyskaya nadezhda), 69-29 (Feyerverk × Bylinnaya) and 72-71 (Privlekatelnaya × Bylinnaya). Examples of the obtained electrophoretic profiles of marker fragments are shown in Figure 1(a), results of the analysis are shown in Table 2.

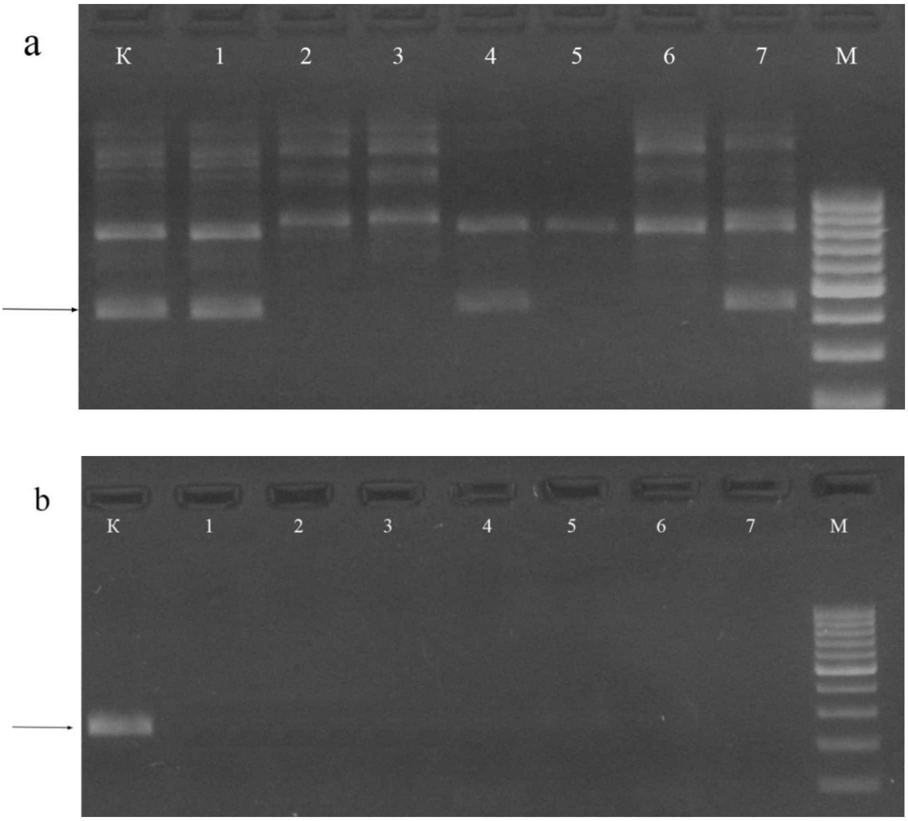

Fig. 1. Electrophoretic profiles of marker fragments OPO-16C (a) and SCAR-R1A (b) *Rpf1* gene in strawberry varieties
K – control (*F. virginiana* subsp. *platypetala*), 1 – Malwina, 2 – Asia, 3 – Chamora Turusi, 4 – Lebedushka, 5 – Flamenco, 6 – Salsa, 7 – Verona, M – Molecular weight marker

The SCAR-R1A marker was identified in selected strawberry forms 61-15 (Bylinnaya × Olimpiyskaya nadezhda), 69-29 (Feyerverk × Bylinnaya) and 72-71 (Privlekatelnaya ×

Bylinnaya). In the analyzed promising strawberry varieties, the SCAR-R1A marker is absent. Examples of the obtained electrophoretic profiles of marker fragments are shown in Figure 1(b), results of the analysis are shown in Table 2.

Table 2. Allelic polymorphism of the *Rpf1* red stele root rot resistance gene in strawberry varieties and hybrid forms (1 – allele is present, 0 – allele is absent)

Genotype	Marker SCAR-R1A	Marker OPO-16C	Putative genotype
Lebedushka	0	1	*rpf1rpf1*
Asia	0	0	*rpf1rpf1*
Chamora Turusi	0	0	*rpf1rpf1*
Clery	0	0	*rpf1rpf1*
Elianny	0	1	*rpf1rpf1*
Flamenco	0	0	*rpf1rpf1*
Florence	0	1	*rpf1rpf1*
Malwina	0	1	*rpf1rpf1*
Monterey	0	1	*rpf1rpf1*
Polka	0	1	*rpf1rpf1*
Salsa	0	0	*rpf1rpf1*
Symphony	0	0	*rpf1rpf1*
Verona	0	1	*rpf1rpf1*
Vima Tarda	0	1	*rpf1rpf1*
56-5	0	1	*rpf1rpf1*
61-15	1	1	*Rpf1rpf1*
69-29	1	1	*Rpf1rpf1*
69-42	0	0	*rpf1rpf1*
72-71	1	1	*Rpf1rpf1*
35-16	0	0	*rpf1rpf1*

According to the obtained data, the analyzed strawberry varieties are characterized by a recessive homozygous state of the *Rpf1* gene: in the varieties Lebedushka, Elianny, Florence, Malwina, Monterey, Polka, Verona, and Vima Tarda is present only the OPO-16C marker; the varieties Asia, Chamora Turusi, Clery, Flamenco, Salsa and Symphony do not have SCAR-R1A and OPO-16C markers. Selected strawberry forms 61-15 (Bylinnaya × Olimpiyskaya nadezhda), 69-29 (Feyerverk × Bylinnaya) and 72-71 (Privlekatelnaya × Bylinnaya) are characterized by a heterozygous genotype for the *Rpf1* gene. Strawberry seedlings 56-5 (Gigantella Maxim × Privlekatelnaya), 69-42 (Feyerverk × Bylinnaya) and 35-16 (922-67 × Maryshka) have a recessive homozygous genotype (*rpf1rpf1*).

4 Conclusion

Thus, as a result of the research, we identified the allelic state of the *Rpf1* gene in 14 promising strawberry varieties of foreign breeding and 6 strawberry selected forms created in the I.V. Michurin FSC. The *Rpf1* gene in a heterozygous state was identified in strawberry hybrid forms 61-15 (Bylinnaya × Olimpiyskaya nadezhda), 69-29 (Feyerverk × Bylinnaya) and 72-71 (Privlekatelnaya × Bylinnaya), which makes it possible to recommend them for involvement in breeding work to create resistant to *P. fragariae* var. *fragariae* strawberry varieties.

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