Marker controlled screening of resistant to red stele root rot (*Rpf1* gene) strawberry selected forms

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Abstract. The results of the analysis of allelic polymorphism of the *Rpf1* red stele root rot resistance gene in strawberry hybrid forms using diagnostic DNA markers were shown. The recessive homozygous genotype (*rpf1rpf1*) was identified in strawberry seedlings 932-29 (*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Feyerverk), 933-4 (*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Rubinovyy kulon), 62- 7, 62-23 (Bylinnaya × Feyerverk), 65-2, 65-15 (Olimpiyskaya nadezhda × Bylinnaya) and 35-16 (922-67 × Maryshka). The heterozygous state of the *Rpf1* gene was identified in strawberry forms 69-29 (Feyerverk × Bylinnaya), 62-41 (Bylinnaya × Feyerverk), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), 65-17, 65-24 (Olimpiyskaya nadezhda × Bylinnaya), which allows them to be used in breeding for resistance to *P. fragariae* var. *fragariae* as valuable initial forms.

1 Introduction

Phytophthora fragariae var. *fragariae* Hickman is the causative agent of red stele root rot, one of the most dangerous strawberry fungal diseases [1, 2]. According to the list of the European and Mediterranean Plant Protection Organization (EPPO), *P. fragariae var. fragariae* has been A2 quarantine pathogen status [3].

Symptoms of red stele root rot of strawberry plants include reddening of the axial cylinder and death of root tips, inhibition of growth, wilting and death of plants [4, 5].

Resistance red stele root rot in strawberry varieties and forms is due to the presence of race-specific oligogens in the genome [6], of which the *Rpf1*, *Rpf2*, and *Rpf3* genes make the greatest contribution to the formation of resistance [7].

Identification of genetic determinants of strawberry resistance to *P. fragariae* var. *fragariae* allows for targeted screening of resistant genotypes using diagnostic DNA markers [8, 9].

The purpose of this study was the analyze allelic polymorphism of the *Rpf1* red stele root rot resistance gene in strawberry hybrid seedlings for identify and involve valuable forms in the breeding programs to create resistant to fungal pathogens strawberry genotypes.

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2 Materials and methods

The studies were carried out in 2020-2021. Biological material was represented by promising strawberry hybrid seedlings obtained at the "I.V. Michurin Federal Scientific Center".

Total genomic DNA of strawberry genotypes was extracted using the DArT method with modifications [10, 11].

Alleles of the *Rpf1* gene in the strawberry genome were identified by DNA analysis using diagnostic markers OPO-16C (5'-TCGGCGGTTC-3') [12] and SCAR-R1A (for 5'-TGCATCATTAATGTAGAAGTCTTT-3', rev 5'-TGATGCGACATACAAAAATATTAG-3') [8].

The SCAR-R1A marker (285 bp amplicon) corresponds to the *Rpf1* resistance allele, and the target fragment is synthesized only if *Rpf1* allele is present in the strawberry genome. The OPO-16C marker (438 bp amplicon) corresponds to the non-functional *rpf1* allele. The combination of these markers makes it possible to identify the allelic state of the *Rpf1* gene in the studied strawberry genotypes [13].

The reaction mix for PCR in total volume of 15 μL contained:

- SCAR-R1A marker: 1.5 μl Taq-buffer, 2.0 mM of each deoxyribonucleotide triphosphate, 2.5 mM magnesium chloride, 0.2 U Taq DNA polymerase, 0.2 μM of each primer and 20 ng of genomic DNA;
- OPO-16C marker: 1.5 μl Taq-buffer, 0.8 mM of each deoxyribonucleotide triphosphate, 2.5 mM magnesium chloride, 0.3 U Taq DNA polymerase, 0.5 μM primer and 100 ng of genomic DNA.

The amplification was performed in T100 Thermal Cycler (BioRad) according to the programs:

- OPO-16C marker: 5 min denaturation at 94°C followed by 36 cycles of 30 s at 94°C, 45 s at 34°C and 60 s at 72°C, followed by a final extension step of 5 min at 72°C;
- SCAR-R1A marker: 3 min denaturation at 94°C followed by 25 cycles of 30 s at 94°C, 45 s at 60°C and 60 s at 72°C, followed by a final extension step of 7 min at 72°C.

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 is identified in strawberry hybrid forms 932-29 (*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Feyerverk), 933-4 (*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Rubinovyy kulon), 62-7, 62-41 (Bylinnaya × Feyerverk), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), 65-17, 65-24 (Olimpiyskaya nadezhda × Bylinnaya) and 69-29 (Feyerverk × Bylinnaya) (Figure 1., Table).

In addition to the target fragment 285 bp, all studied strawberry genotypes also contain additional fragments of the OPO-16C marker. Amplification of additional fragments of the OPO-16C marker is also confirmed by other researchers. These fragments are synthesized in any strawberry forms and are not diagnostic signs of the allelic state of the *Rpf1* gene [13, 14].

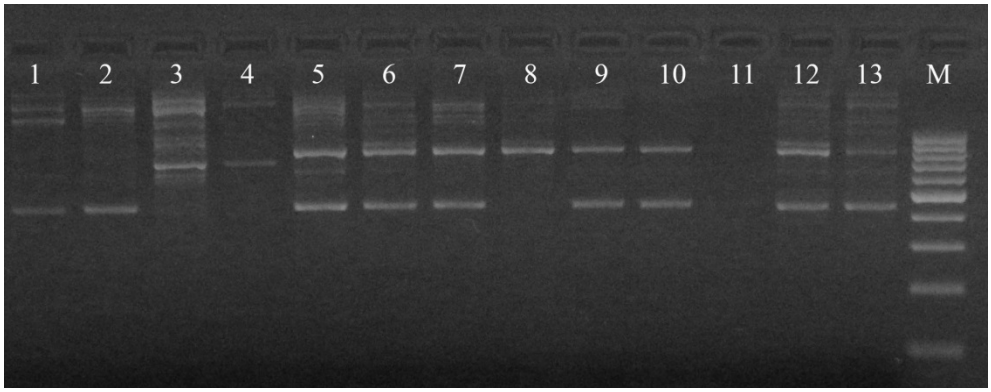

Fig. 1. Electrophoretic profiles of marker fragments (OPO-16C) of the *Rpf1* gene in strawberry hybrid forms
1 – 933-4, 2 – 932-29, 3 – 62-23, 4 – 65-2, 5 – 69-29, 6 – 62-7, 7 – 62-41, 8 – 65-15, 9 – 72-24, 10 – 72-71, 11 – 35-16, 12 – 65-17, 13 – 65-24, M – Molecular weight marker

The marker SCAR-R1A was identified in strawberry seedlings 69-29 (Feyerverk × Bylinnaya), 62-41 (Bylinnaya × Feyerverk), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), 65-17, 65-24 (Olimpiyskaya nadezhda × Bylinnaya) (Figure. 2., Table.).

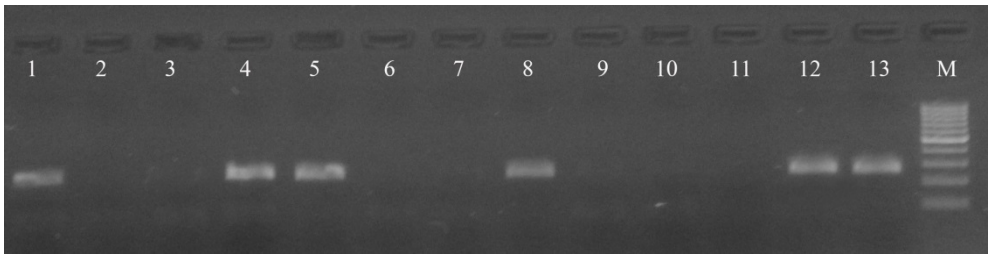

Fig. 2. Electrophoretic profiles of marker fragments (SCAR-R1A) of the *Rpf1* gene in strawberry hybrid forms
1 – 69-29, 2 – 933-4, 3 – 932-29, 4 – 62-41, 5 – 72-24, 6 – 62-7, 7 – 62-23, 8 – 72-71, 9 – 65-2, 10 – 65-15, 11 – 35-16, 12 – 65-17, 13 – 65-24, M – Molecular weight marker

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

Genotype	Origin	Marker SCAR-R1A	Marker OPO-16C	Putative genotype
933-4	*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Rubinovyy kulon	0	1	*rpf1rpf1*
932-29	*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Feyerverk	0	1	*rpf1rpf1*
69-29	Feyerverk × Bylinnaya	1	1	*Rpf1rpf1*
62-7	Bylinnaya × Feyerverk	0	1	*rpf1rpf1*
62-23		0	0	*rpf1rpf1*
62-41		1	1	*Rpf1rpf1*
72-24	Privlekatelnaya × Bylinnaya	1	1	*Rpf1rpf1*
72-71		1	1	*Rpf1rpf1*
65-2	Olimpiyskaya nadezhda × Bylinnaya	0	0	*rpf1rpf1*
65-15		0	0	*rpf1rpf1*
65-17		1	1	*Rpf1rpf1*
65-24		1	1	*Rpf1rpf1*
35-16	922-67 × Maryshka	0	0	*rpf1rpf1*

The obtained data indicate the heterozygous state of the *Rpf1* gene in strawberry forms 62-41 (Bylinnaya × Feyerverk), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), 65-17, 65-24 (Olimpiyskaya nadezhda × Bylinnaya) and 69-29 (Feyerverk × Bylinnaya) (markers OPO-16C and SCAR-R1A are present in the genotype). Strawberry seedlings 932-29 (*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Feyerverk), 933-4 (*F. virginiana* subsp. *platypetala* (Rydb.) Staudt × Rubinovyy kulon) and 62-7 (Bylinnaya × Feyerverk) are characterized by the recessive homozygous state of the *Rpf1* (only marker OPO-16C is present in the genotype). Strawberry seedlings 62-23 (Bylinnaya × Feyerverk), 65-2, 65-15 Olimpiyskaya nadezhda × Bylinnaya) and 35-16 (922-67 × Maryshka) also are characterized by the recessive homozygous state of the *Rpf1* (both markers are absent in the genotype).

Other researchers have also identified selected strawberry forms with the *Rpf1* resistance allele in the genome: CPRO 90025, MD683, MDUS 3184 and Yalova-15 (heterozygous genotype), CPRO 88239, CPRO 88246, CPRO 88275 and Yalova-4 (homozygous genotype) [8]. The identification of homozygous for the *Rpf1* gene strawberry forms is of great practical importance, making it possible to obtain up to 100% resistant seedlings when used in hybridization. The use of homozygous forms in crossing will theoretically avoid the need for phenotypic or genotypic analysis of hybrid seedlings for a selective trait, which will reducing the analysis time, labor and financial costs. [15].

Analysis of the origin of the studied strawberry hybrid seedlings confirmed the data of molecular genetic testing. Strawberry seedlings 69-29 (Feyerverk × Bylinnaya), 62-41 (Bylinnaya × Feyerverk), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), 65-17, 65-24 (Olimpiyskaya nadezhda × Bylinnaya), characterized by the presence of both markers in the genome and, therefore, the heterozygous state of the *Rpf1* gene, were isolated in crossing combinations, where the donor of the functional *Rpf1* allele is the Bylinnaya variety, and other parental genotypes (Olimpiyskaya nadezhda, Privlekatelnaya and Feyerverk) do not have the functional *Rpf1* allele [16]. In this regard, strawberry hybrids with the identified *Rpf1* gene can only have a heterozygous genotype (*Rpf1rpf1*). In the future, the selected hybrid seedlings will be crossed to obtain strawberry forms homozygous for the *Rpf1* gene.

4 Conclusion

Thus, in the result of the research we analyzed the allelic polymorphism of the *Rpf1* gene in strawberry hybrid seedlings. The perspective strawberry forms (69-29 (Feyerverk × Bylinnaya), 62-41 (Bylinnaya × Feyerverk), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), 65-17, 65-24 (Olimpiyskaya nadezhda × Bylinnaya)), characterized by resistance to red stele root rot (putative genotype *Rpf1rpf1*) were identified.

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