Marker-assisted screening of promising forms in the strawberry breeding

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Abstract. The results of the use diagnostic DNA-markers in the breeding of strawberry (*Fragaria* × *ananassa* Duch.) were shown. The carriers of target alleles of red stele root rot resistance (*F. virginiana* Duch. ssp. *platypetala*, *Bylinnaya*, 69-29 (*Feyerverk* × *Bylinnaya*), 72-24 and 72-71 (*Privlekatelnaya* × *Bylinnaya*)), anthracnose resistance (*Borovitskaya*, *Sudarushka*, *Elianny*, *Troubadour*, 933-4 (*F. virginiana* Duch. ssp. *platypetala* × *Rubinovyy kulon*)), high mesifurane content in fruits (*F. orientalis* Los., *F. moschata* Duch., *F. virginiana* Duch. ssp. *platypetala*, *Lastochka*, *Torpeda*, *Flora*, *Samson*, 932-29 (*F. virginiana* Duch. ssp. *platypetala* × *Feyerverk*), 56-5 (*Gigantella* × *Privlekatelnaya*)) and γ -decalactone content in fruits (*F. orientalis* Los., *F. moschata* Duch., *F. ovalis* Rydb., *Bylinnaya*, *Kupchikha*, *Sonata*, *Vima Tarda*) were identified. These genotypes are valuable initial forms for involvement in the breeding process to improve the strawberry assortment.

1 Introduction

Strawberry is the most widely cultivated berry crops. Strawberry plantations are located in 78 countries of the world and the harvest of strawberry fruits exceeds 2/3 of the berry world production [1-3]. However, in current conditions of climate destabilization, massive development of diseases of various etiologies and increased attention to the quality of the berry products, many strawberry varieties do not sufficiently meet the market requirements. In this connection, it is necessary to carry out targeted breeding work in order to create strawberry genotypes characterized by a complex of such traits as resistance to unfavorable factors of growing conditions, high stable productivity and commodity consumer qualities of fruits [4, 5].

A necessary condition for the implementation of the problem is the identification of sources and donors of loci (genes and QTLs) of valuable traits, and attraction and creation of polymorphic source forms. An important stage in the strawberry breeding is the identification of initial parental forms for hybridization. They should not only be characterized by a complex of significant breeding traits, but also, to a certain extent, transmit them to their offspring, which makes it possible to increase the efficiency of creating valuable genotypes.

One of the topical directions for intensifying the process of creating new forms is the combination of classical breeding methods of with breeding technologies based on DNA

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markers (marker assisted selection). The advantage of DNA marking technology is to assess the presence of significant traits not by their phenotypic expression, which is formed under the influence of environmental conditions, but directly by the presence in the genome of target alleles. In addition, the use of molecular DNA markers allows detecting DNA polymorphism, establishing genetic relationships and the origin of varieties and forms, as well as identifying new genes and QTLs. [6-9].

Strawberry (*F. × ananassa* Duch.) is a difficult object for molecular genetic analysis, which is due to the combination of several basic genomes in one genotype, a high level of ploidy (8x) and polygenic determination of many valuable traits. However, the active development of technologies of molecular genetic analysis of the genome has made it possible to deepen knowledge of the mechanisms of determination and inheritance of a number of strawberry significant traits and to map some candidate genes and QTLs [10-12].

Currently, DNA markers are most actively used to identify genetic diversity, mapping, and genetic passportization of strawberry varieties and forms [13-15]. The use of molecular markers in strawberry breeding for the identification of significant trait genes will increase the efficiency of the strawberry breeding, reduce the time to identify donor qualities of genotypes and select the initial parental forms for hybridization.

2 Materials and Methods

The studies were carried out in 2020-2021. Biological material was represented by wild species of genus *Fragaria* L., strawberry varieties of Russian and foreign breeding, and promising hybrid seedlings, obtained at the FSSI "I.V. Michurin Federal Scientific Center".

The promising strawberry genotypes were identified with the DNA markers: marker SCAR-R1A – *Rpfl* gene (red stele root rot resistance) [16], marker STS-Rca2_240 – *Rca2* gene (anthracnose resistance) [17], marker FaOMT-SI/NO – *FaOMT* gene (mesifurane content in fruits) [12] and marker FaFAD1-F/R – *FaFAD1* gene (γ-decalactone content in fruits) [18] (Table. 1.).

Table 1. DNA markers used for molecular genetic analysis

Gene	Marker	Primer sequence (5'→3')	Product length (bp)
<i>FaFAD1</i>	FaFAD1-F/R	F 5'-CGGGATTAATGGTTTTGTTGTTGACCGACC-3' R 5'-GTAGAAGAGAGAGACCAAGACGAG-3'	500
<i>Rpfl</i>	SCAR-R1A	F 5'-TGCATCATTAATGTAGAAGTCTTT-3' R 5'-TGATGCGACATACAAAAATATTAG-3'	285
<i>FaOMT</i>	FaOMT-SI/NO	F 5'-CGATCATTTTCGAAAAGGACTA-3' R 5'-AAGCAGGGTTAGTTGTGGAGA-3'	217, 248
<i>Rca2</i>	STS-Rca2_240	F 5'-GCCACGTCAGTCAAAATTCAA-3' R 5'-TCATGGACAGTGGTCTCAGC-3'	240

Reaction mix in final volume 15 µl containing 1.5 µl Taq-buffer, 0.2 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.

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, USA).

3 Results and Discussion

In the "I.V. Michurin Federal Scientific Center" active research by the molecular genetic

analysis of the initial forms and marker-assisted selection of strawberries are underway. For this purpose, genomic DNA was isolated and a DNA collection was created, including octoploid (8x) species *F. virginiana* ssp. *platypetala* and *F. ovalis*, hexaploid (6x) species *F. moschata* and tetraploid (4x) species *F. orientalis*, strawberry varieties of Russian breeding (Bylinnaya, Urozhaynaya CGL, Yarkaya, Karnaval, Solovushka, Kokinskaya zarya, Tsaritsa, Lastochka, Rusich, Feyerverk, Privlekatelnaya, Torpeda, Flora, etc.) and foreign breeding (Aprica, Vima Kimberly, Elsanta, Red Gauntlet, Vima Tarda, Polka, Elianny, Vima Zanta, Symphony, etc.), and strawberry promising selected and elite forms of interspecific origin (26-5 (Rubinovyy kulon × 298-19-9-43), 933-4 (*F. virginiana* ssp. *platypetala* × Rubinovyy kulon), 35-16 (922-67 × Maryshka), etc.) and strawberry genotypes intervarietal origin (69-29 (Feyerverk × Bylinnaya), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), etc.).

The marker STS-Rca2_240, linked to the *Rca2* anthracnose resistance gene in the studied strawberry collection was identified in the Russian varieties Borovitskaya and Sudarushka, and foreign varieties Elianny and Troubadour, and selected form 933-4 (*F. virginiana* ssp. *platypetala* × Rubinovyy kulon) (Figure. 1., Table. 2.).

Fig. 1. Electrophoresis profile of marker STS-Rca2_240 at strawberry genotypes

Where: 1 – Vima Tarda, 2 – 35-16, 3 – Symphony, 4 – Flora, 5 – Zenit, 6 – Karmen, 7 – Sonata, 8 – Bylinnaya, 9 – 26-5, 10 – 933-4, 11 – Sudarushka, 12 – Vima Zanta, 13 – Lastochka, 14 – Rusich, M – Molecular weight marker

The marker SCAR-R1A, linked to the *Rpfl* red stele root rot resistance gene was identified in strawberry variety Bylinnaya, strawberry selected forms 69-29 (Feyerverk × Bylinnaya), 72-24, 72-71 (Privlekatelnaya × Bylinnaya), and octoploid wild species *F. virginiana* ssp. *platypetala*, which can be used as a source in strawberry marker-assisted selection for red stele root rot resistance (Figure. 2., Table. 2.).

Fig. 2. Electrophoresis profile of marker SCAR-R1A at strawberry genotypes

Where: 1 – Karnaval, 2 – 928-12, 3 – Lastochka, 4 – 72-71, 5 – Privlekatelnaya, 6 – 35-16, 7 – 932-29, 8 – Polka, 9 – Bylinnaya, M – Molecular weight marker

Table 2. Allelic diversity of the Rca2 anthracnose resistance, Rpf1 red stele root rot resistance, and FaOMT and FaFAD1 fruit aroma genes in strawberry genotypes

№	Genotype	Rpf1	Rca2	FaOMT		FaFAD1
		285 bp	240 bp	217 bp	248 bp	500 bp
1	<i>F. orientalis</i> Los.				+	+
2	<i>F. moschata</i> Duch.				+	+
3	<i>F. virginiana</i> Duch. ssp <i>platypetala</i>	+			+	
4	<i>F. ovalis</i> Rydb.			+		+
5	Borovitskaya		+		+	
6	Bylinnaya	+		+		+
7	Zenit				+	
8	Karnaval				+	
9	Krymchanka 87				+	
10	Kupchikha			+		+
11	Lastochka				+	
12	Neznakomka			+	+	
13	Olimpiyskaya nadezhda			+		
14	Privlekatelnaya			+	+	
15	Rusich			+	+	
16	Studencheskaya				+	
17	Sudarushka		+	+		
18	Torpeda				+	
19	Flora				+	
21	Elianny		+		+	
22	Karmen				+	
24	Ostara			+	+	
25	Polka			+	+	
26	Quicky			+		
27	Samson				+	
28	Sonata			+	+	+
29	Symphony			+	+	
30	Troubadour		+	+	+	
31	Vima Tarda			+	+	+
32	Vima Zanta				+	
33	933-4		+			
34	35-16					
35	26-5			+	+	
36	69-29	+		+	+	
37	72-24	+		+	+	
38	72-71	+		+	+	
39	928-12			+	+	
40	932-29				+	
41	56-5				+	

Using the marker FaOMT-SI/NO, the functional allele of the *FaOMT* gene, which determines the high mesifurane content in fruits, was identified in species *F. virginiana* ssp *platypetala*, *F. orientalis* and *F. moschata*, strawberry varieties Rusich, Neznakomka, Lastochka, Torpeda, Flora, Samson, Polka, etc., and strawberry selected forms 56-5 (Gigantella × Privlekatelnaya), 928-12 (298-19-9-43 × Privlekatelnaya), etc. The functional allele of the *FaOMT* gene in the homozygous state was detected in wild species *F. virginiana* ssp *platypetala*, *F. orientalis* and *F. moschata*, and strawberry hybrid of interspecific origin 932-29 (*F. virginiana* ssp. *platypetala* × Feyerverk) and strawberry genotypes intervarietal origin (Flora, Zenit, Borovitskaya, Krymchanka 87, Lastochka, Studencheskaya, Karnaval, Torpeda, Samson, Vima Zanta, Elianny, Karmen, 56-5 (Gigantella × Privlekatelnaya)) (Figure. 3., Table. 2.).

Fig. 3. Electrophoresis profile of marker FaOMT-SI/NO at strawberry genotypes

Where: 1 – *F. orientalis*, 2 – 56-5, 3 – 26-5, 4 – *F. moschata*, 5 – Vima Zanta, 6 – Lastochka, 7 – Krymchanka 87, 8 – Ostara, 9 – Quicky, 10 – Polka, 11 – Torpeda, 12 – Flora, 13 – Samson, M – Molecular weight marker

The marker FaFAD1-F/R, linked to the *FaFAD1* gene (high γ -decalactone content in fruits), was identified in strawberry varieties Bylinnaya, Kupchikha, Sonata and Vima Tarda, and wild species *F. orientalis*, *F. moschata* and *F. ovalis* (Figure. 4., Table. 2.).

Fig. 4. Electrophoresis profile of marker FaFAD1-F/R at strawberry genotypes

Where: 1 – 72-24, 2 – Karnaval, 3 – 69-29, 4 – Zenit, 5 – Sonata, 6 – Ostara, 7 – Bylinnaya, 8 – 26-5, 9 – 928-12, 10 – Sudarushka, 11 – Flora, 12 – Kupchikha, 13 – Vima Zanta, 14 – Vima Tarda, M – Molecular weight marker

4 Conclusion

Thus, as a result of molecular genetic analysis of the strawberry genetic collection, promising forms-carriers of the target alleles of genes of selection-significant traits were identified: *F. virginiana* ssp. *platypetala*, Bylinnaya, 69-29, 72-24, 72-71 (red stele root rot resistance); Borovitskaya, Sudarushka, Elianny, Troubadour, 933-4 (anthracnose resistance); *F. virginiana* ssp. *platypetala*, *F. orientalis*, *F. moschata*, Lastochka, Torpeda, Flora, Samson, 932-29, 56-5, etc. (high mesifurane content in fruits); *F. orientalis*, *F. ovalis*, *F. moschata*, Bylinnaya, Kupchikha, Sonata, Vima Tarda (high γ -decalactone content in fruits), involvement in hybridization of which will accelerate the process of creating new strawberry forms with specified parameters of traits.

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