

Molecular genetic analysis of strawberry genotypes for the *FaOMT* fruit aroma gene

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Abstract. The results of the analysis of allelic polymorphism of strawberry varieties and forms for the *FAOMT* fruit aroma gene were shown. The non-functional allele *FaOMT*⁻ in the homozygous state was detected in strawberry variety Quicky. Heterozygous genotype (*FaOMT*⁺*FaOMT*⁻) was identified in the strawberry varieties Feyerverk, Ostara, Polka and Symphony, and selected forms 26-5 and 928-12. The functional allele *FaOMT*⁺ gene (*FaOMT*⁺) in the homozygous state (*FaOMT*⁺*FaOMT*⁺ genotype) was detected in strawberry varieties Borovitskaya, Kubata, Troitskaya, Tsaritsa, Yarkaya, Korona and Vima Kimberly, and selected forms 932-29 and 298-19-9-43, which allows us to be used as valuable initial forms in breeding for fruit aroma.

1 Introduction

Strawberry is one of the most widespread berry crops in the world. Its popularity is due to such traits as early harvest maturity, dessert taste, aroma and rich biochemical composition of the fruit [1, 2]. It should be noted that the strawberry breeding is mainly aimed at improving the commercial qualities of fruits, productivity and plant resistance to unfavorable biotic factors, while the improvement of such consumer qualities as the biochemical composition and aroma of fruits was carried out insufficiently, which led to a decrease in these traits in many widely cultivated strawberry varieties [3, 4].

The pleasant aroma of strawberry fruits is due to the content of a large amount of volatile organic compounds. In strawberry fruits over 360 volatile compounds reported [5-7]. The most important components of the aromatic profile of strawberry fruits are about 20 volatile compounds. These include 2,5-dimethyl-4-hydroxy-3 (2H)-furanone (furanol) and 4-methoxy-2,5-dimethyl-3-furanone (mesifurane). Furanol and mesifurane add sweet and caramel notes to the strawberry aroma [8, 9].

The furanones content in strawberry fruits are dependent on the genotype, the degree of fruit maturity and environmental conditions during their growth and development [3, 10, 11]. Moreover, unlike most quantitative traits of strawberry, the formation of which is due to polygenic effects, the concentration of 4-methoxy-2,5-dimethyl-3-furanone in fruits is controlled by the dominant *FaOMT* gene. The *FaOMT* gene has two allelic states. Allele

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FaOMT⁺ determines a high level of accumulation of mesifurane in strawberry fruits. Allele *FaOMT*⁻ is inactive (non-functional). The active allele (*FaOMT*⁺) differs from the inactive allele (*FaOMT*⁻) by the presence in the promoter of several SNPs (Indel) containing potential regulatory elements – the E-box/RRE motif, the MYBL motif, and the ABRE/ACGT motif. [12]. Monogenic control of the mesifurane content in strawberry fruits is also confirmed by the RNA interference, which showed an almost complete absence of mesifurane in strawberry fruits during repression of the *FaOMT* transcripts [13]. Identification of the main determinants of this trait allows screening of promising forms based on molecular markers [12, 14].

The purpose of study was to study the allelic diversity of the *FaOMT* fruit aroma gene in strawberry varieties in order to identify promising genotypes for involvement in the strawberry breeding to improve the aroma of fruits.

2 Methods

The studies were carried out in 2019-2020. Biological material was represented by strawberry varieties and selected forms from genetic collection of the FSSI "I.V. Michurin Federal Science Center".

Total genomic DNA of strawberry genotypes was extracted using the Diversity Arrays Technology P/L (DArT) protocol with modifications [15, 16].

The alleles of *FaOMT* gene were identified with the primers *FaOMT*-SI/NO-F (5'-CGATCATTTTCGAAAAGGACTA-3') and *FaOMT*-SI/NO-R (5'-AAGCAGGGTTAGTTGTGGAGA-3'). The *FaOMT*⁺ allele on the electrophoregram corresponds to a 248 bp fragment, the *FaOMT*⁻ allele corresponds to a 214 bp fragment [10].

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.

The polymerase chain reaction was performed in T100 Thermal Cycler (BioRad). PCR conditions were as follows: 3 min denaturation at 95°C followed by 10 cycles of 30 s at 95°C, 30 s at 60°C (-0.5 °C/cycle), and 45 s at 72 °C, then 25 cycles of 30 s at 95 °C, 30 s at 55°C, and 45 s at 72°C, followed by a final extension step of 5 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

According to the research the functional allele *FaOMT*⁺ was detected in 15 forms (93.7%) out of 16 analyzed genotypes. The *FaOMT*⁻ allele was identified in 43.7% analyzed forms. The *FaOMT*⁺*FaOMT*⁻ genotype was identified in 37.5% forms. The *FaOMT*⁺*FaOMT*⁺ genotype was detected in 56.2% forms. The homozygous state of non-functional allele was identified in foreign strawberry variety Quicky (the example of electrophoresis profile of markers *FaOMT*-SI/NO is shown in Fig. 1, the results are shown in Tab. 1.).

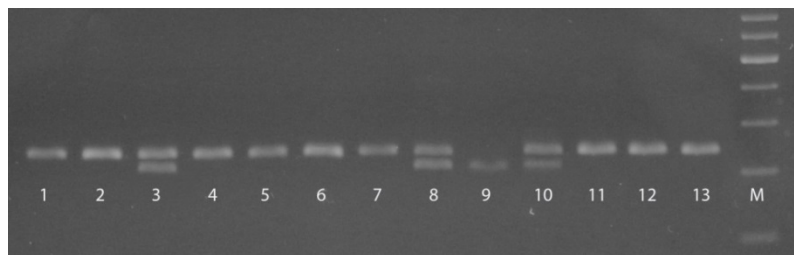


Fig. 1. Electrophoresis profile of markers FaOMT-SI/NO at strawberry genotypes
 1 – Borovitskaya, 2 – 298-19-9-43, 3 – 26-5, 4 – Korona, 5 – Vima Kimberly, 6 – Yarkaya,
 7 – Troitskaya, 8 – Ostara, 9 – Quicky, 10 – Polka, 11 – 932-29, 12 – Kubata, 13 – Tsaritsa,
 M – Molecular weight marker

Table 1. Allelic polymorphism of *FaOMT* gene in strawberry varieties and forms
 (1 – allele is presence, 0 – allele is absence)

№	Genotype	<i>FaOMT</i>	
		217 bp	248 bp
1	Borovitskaya (Nadezhda × Red Gauntlet)	0	1
2	Kubata (Kubenskaya × Holiday)	0	1
3	Troitskaya (<i>F.</i> × <i>ananassa</i> Duch. × <i>F. moschata</i> Duch.)	0	1
4	Feyerverk (Zenga Zengana × Redcoat)	1	1
5	Tsaritsa (Venta × Red Gauntlet)	0	1
6	Yarkaya (Zenga Zengana × Redcoat)	0	1
7	Korona (Tamella × Induka)	0	1
8	Ostara (Red Gauntlet × Masherahs Daurernte)	1	1
9	Polka (Unduka × Sivetta)	1	1
10	Quicky (CIVN251)	1	0
11	Symphony (Rhapsody × Holiday)	1	1
12	Vima Kimberly (Gorella × Chandler)	0	1
13	298-19-9-43 [(<i>F. orientalis</i> Los. × <i>F. moschata</i> Duch.) × <i>F. ananassa</i> Duch.]	0	1
14	26-5 (<i>F. ananassa</i> Duch. × [(<i>F. orientalis</i> Los. × <i>F. moschata</i> Duch.) × <i>F. ananassa</i> Duch.]	1	1
15	928-12 [(<i>F. orientalis</i> Los. × <i>F. moschata</i> Duch.) × <i>F. ananassa</i> Duch.]	1	1
16	932-29 (<i>F. virginiana</i> Duch. ssp. <i>platypetala</i> × <i>F. ananassa</i> Duch.)	0	1

In most of the analyzed strawberry varieties (58.3 %), the active allele of the *FaOMT* gene is present in a homozygous form. It should be noted that out of six strawberry varieties of Russian breeding, the homozygous genotype for the *FaOMT* gene (*FaOMT*+*FaOMT*+) was identified in five genotypes (Borovitskaya, Kubata, Troitskaya, Tsaritsa, Yarkaya), while among 6 varieties of foreign breeding – only in two forms (Netherlands’ strawberry varieties Korona and Vima Kimberly). More widespread of the heterozygous genotype of the *FaOMT* gene in foreign strawberry forms also shown in other studies [17, 18]. The prevalence of the *FaOMT*+*FaOMT*- genotype in foreign strawberry varieties can be explained by their genetic proximity due to the active use of several initial forms in hybridization, which presumably could be a donor of the inactive *FaOMT*- allele.

Among the analyzed selected forms of strawberry, homozygous state of the *FaOMT*+ allele was identified in hybrids 298-19-9-43 and 932-29. Selected forms 26-5 and 928-12 have the *FaOMT*+*FaOMT*- genotype.

Thus, as a result of the research, we studied the allelic polymorphism of the *FaOMT* gene, which determines the content of 4-methoxy-2,5-dimethyl-3-furanone (mesifurane) in strawberry fruits. Valuable sources of increased concentration of mesifurane in fruits are strawberry varieties Borovitskaya, Kubata, Troitskaya, Tsaritsa, Yarkaya, Korona and Vima Kimberly, and hybrid forms 932-29 and 298-19-9-43 (*FaOMT*+*FaOMT*+ genotype).

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