Polymorphism of the FaOMT and FaFAD1 genes for fruit flavor volatiles in strawberry varieties and wild species from the genetic collection of the Michurin Federal Research Center

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Полиморфизм сортов и дикорастущих видов земляники генетической коллекции Федерального научного центра им. И.В. Мичурина по генам аромата плодов FaOMT и FaFAD1

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polypolmorphism of the FaOMT and FaFAD1 genes in strawberry from the collection of the Michurin FRC

Introduction

Strawberry (Fragaria × ananassa Duch.) is the most popular and economically important berry crop characterized by high taste and aroma of the fruits (Hummer, Hancock, 2009; Vandendriessche et al., 2013). Until recently, fruit aroma was not considered significant; therefore, many highly productive commercial varieties have feeble fruit aroma (Ulrich, Olbricht, 2016; Bianchi et al., 2017). Currently, due to the insistence on high standards, not only the taste but also the aroma of fruit, more attention is paid to creating varieties with improved fruit aroma (Ulrich, Olbricht, 2011; Zorrilla-Fontanesi et al., 2012).

Valuable source materials for strawberry breeding, including the breeding for fruit aroma, are wild species of the genus Fragaria L. Introggression of genes from wild strawberry species into the germ plasm of cultivated varieties F. × ananassa Duch. is expected to give rise to whole new genetic material and to expand the genetic polymorphism of breeding populations and the range of variation of traits, contributing to the acceleration of strawberry breeding (Hancock et al., 2010; Finn et al., 2013).

The aromatic profile of strawberry fruits is highly complex. It includes more than 350 volatile compounds: esters, furanones, terpenes, aldehydes, ketones, alcohols, sulfur compounds, etc. (Aharoni et al., 2004; Jetti et al., 2007; Schwab et al., 2008). The most important components of strawberry fruit aroma are furanones; in particular, furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) and its derivative mesifurane (2,5-dimethyl-4-methoxy-3(2H)-furanone). Furaneol and mesifurane contribute to fruit caramel aroma. The more furanones are contained in strawberry fruits, the sweeter is their aroma (Lavid et al., 2002; Raab et al., 2006). Another compound important for strawberry fruit aroma is γ-decalactone. This volatile contributes to fruity, sweet, or peachy aroma (Jouquand et al., 2002; Schwab et al., 2008). The concentrations of mesifurane and γ-decalactone in strawberry fruit are highly dependent on the genotype, environmental conditions, and the degree of fruit maturity (Ménager et al., 2004; Jetti et al., 2007; Olbricht et al., 2008; Siegmund et al., 2010). Moreover, unlike most components of the aromatic complex of strawberry fruits, whose biosynthesis is determined quantitatively, the contents of mesifurane and γ-decalactone are controlled by the dominant FaOMT and FaFAD1 genes, respectively. Therefore, functional DNA markers can be applied to effective screening of genotypes with high levels of the target traits, which allows highly reliable identification of carriers of target gene alleles at early developmental stages (Zorrilla-Fontanesi et al., 2012; Chambers et al., 2014; Sánchez-Sevilla et al., 2014).

The objectives of this study were the molecular genotyping of plants of the genus Fragaria L. for the FaOMT and FaFAD1 fruit flavor volatile genes, analysis of polymorphism for the loci of interest, and identification of valuable genotypes in breeding for fruit aroma.

Materials and methods

Experiments were conducted with wild species and commercial varieties of strawberry from the genetic collection of the Michurin Federal Research Center, including 4 wild species of the genus Fragaria L., Kupchikha variety (F. × anashata Kantor.), and 38 strawberry varieties (Fragaria × ananassa Duch.), of which 22 genotypes were bred in Russia and 16 genotypes, outside Russia (Table 1).

Total genomic DNA was extracted from fresh leaves using the Diversity Arrays Technology P/L (DArT, 2014) modified as in (Luk’yanchuk et al., 2018).

To assess the FaOMT allelic state, the codominant marker FaOMT-SI/NO (Zorrilla-Fontanesi et al., 2012) was used. The FaFAD1 gene was identified with the dominant marker FaFAD1 (Chambers et al., 2014). Primers used in this study were synthesized by Syntol (Russia). Sequences:

- FaOMT-SI/NO F 5’-CGATCATTTCGAAAAGGAC TA-3’, R 5’-AAAGCAGGGTTAGTTGTGGAGA-3’;
- FaFAD1 F 5’-CGGGAATATGGTTTTGTTGACC GACC-3’, R 5’-GTAGAAGAGAGAACCCAGACGAG-3’.

PCR reactions were conducted in 15 μL of the amplification mixture containing 20 ng of genomic DNA, 0.2 mM of each dNTP, 2.5 mM MgCl₂, 0.2 μM each primer, 0.2 U of Taq DNA polymerase, and 1.5 μL of PCR-buffer (+(NH₄)₂SO₄, -KCl). All components were purchased from Thermo Fisher Scientific.

The amplification was performed in a T100 Thermal Cycler (BioRad). The PCR conditions for the FaOMT-SI/NO marker were as described by Cruz-Rus et al. (2017): predenaturation at 95 °C for 3 min followed by 10 cycles of 95 °C for 30 s, 60 °C (-0.5 °C/cycle) for 30 s, and 72 °C for 45 s; then 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s; postextension at 72 °C for 5 min.

PCR conditions for the FaFAD1 marker were as described by Chambers et al. (2014): predenaturation at 94 °C for 4 min followed by 25 cycles of 94 °C for 30 s, 56 °C for 30 s, 72 °C for 30 s; postextension at 72 °C for 10 min.

1 The term “strawberry fruit” denotes the consumable overgrown juicy receptacle with numerous seeds (achenes) embedded on its surface, which is classified as an aggregate accessory fruit.
### Table 1. Analyzed wild species and varieties of strawberry

| No. | Genotype                          | Origin/Originator                                                                 |
|-----|-----------------------------------|-----------------------------------------------------------------------------------|
| 1   | *F. orientalis* Los.              | Primorsky Kray, Russia                                                            |
| 2   | *F. moschata* Duch.               | European Russia                                                                   |
| 3   | *F. ovalis* Rydb.                 | British Columbia, Canada                                                           |
| 4   | *F. virginiana* Duch. ssp. platypetala |                                                                                  |
| 5   | Alyona                            | All-Russia Horticultural Institute for Breeding Agrotechnology and Nursery, Russia |
| 6   | Vityaz                            |                                                                                  |
| 7   | Rusich                            |                                                                                  |
| 8   | Solovushka                        |                                                                                  |
| 9   | Zenit                             |                                                                                  |
| 10  | Sudarushka                        |                                                                                  |
| 11  | Kupchikha                         | Kokino Station of the All-Russia Horticultural Institute for Breeding, Agrotechnology, and Nursery, Russia |
| 12  | Studencheskaya                    |                                                                                  |
| 13  | Krymchanka 87                     | The Nikita Botanical Garden (National Scientific Center of the Russian Academy of Sciences), Republic of Crimea, Russia |
| 14  | Lastochka                         | Michurin Federal Research Center, Russia                                           |
| 15  | Privlekatelnaya                   |                                                                                  |
| 16  | Flora                             |                                                                                  |
| 17  | Festivalnaya apomikt              |                                                                                  |
| 18  | Divmaya                           | Institute for Engineering and Environmental Problems in Agricultural Production, Russia |
| 19  | Tsarskoleskaya                    |                                                                                  |
| 20  | Festivalnaya                      | N.I. Vavilov All-Russia Institute of Plant Genetic Resources, Russia              |
| 21  | Torpeda                           | Sverdlovsk Breeding Station of Horticulture of the All-Russia Horticultural Institute for Breeding, Agrotechnology, and Nursery, Russia |
| 22  | Bilynnaya                         | Krym Experimental Breeding Station of the N.I. Vavilov All-Russia Institute of Plant Genetic Resources, Russia |
| 23  | Karnaval                          | Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Russia, Govorova G.F. |
| 24  | Olimpiyskaya nadezhda             |                                                                                  |
| 25  | Bogema                            |                                                                                  |
| 26  | Neznakomka                        | All-Russia Horticultural Institute for Breeding Agrotechnology and Nursery, Russia, Popova I.V. |
| 27  | Girlyanda                         | Poisk Company, Russia                                                             |
| 28  | Troubadour                        | United Kingdom                                                                    |
| 29  | Red Gauntlet                      | Scotland                                                                         |
| 30  | Festivalnaya romashka             | Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine, Ukraine |
| 31  | Polka                             | Plant Research International – WUR, Netherlands                                    |
| 32  | Gigantella Maxim                  | Netherlands                                                                       |
| 33  | Sonata                            |                                                                                  |
| 34  | Vima Tarda                        | Vissers International BV, Netherlands                                             |
| 35  | Vima Zanta                        |                                                                                  |
| 36  | Barildaun                         | USA                                                                              |
| 37  | Marshall                          |                                                                                  |
| 38  | Samson                            |                                                                                  |
| 39  | Karmen                            | Czech Republic                                                                    |
| 40  | Maryshka                          |                                                                                  |
| 41  | Symphony                          | Mynfield Research Services Ltd, United Kingdom                                    |
| 42  | Elianny                           | Gebr. Vissers, Netherlands                                                        |
| 43  | Tokado                            | Japan                                                                            |
The amplification products were resolved in 2% agarose gel and visualized by ethidium bromide staining. GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific) was used as a molecular weight marker.

Results and discussion

The mesifurane content in strawberry fruit is controlled by the FaOMT gene, which is mapped to the distal region of the long arm of the chromosome VII-F1. The difference between the functional and nonfunctional alleles of the FaOMT gene is due to several single-nucleotide insertions/deletions (indels) in the promoter region of the gene, whose sizes total 30 bp. Primers FaOMT-SI/NO flanking the region with indels allow identification of the active (fragment of 248 bp) and inactive (fragment of 217 bp) FaOMT alleles (Zorrilla-Fontanesi et al., 2012). The effect of the FaOMT gene on mesifurane concentration was analyzed in a 232 × 1392 segregating population, where both parent forms were characterized by high mesifurane concentrations in the fruit. Statistical analysis of the results confirmed the participation of a single locus in the formation of the trait (expected 3:1 ratio, p = 0.36). We also analyzed the expression level of FaOMT in ripe fruits of forms with contrasting mesifurane contents. This analysis showed high FaOMT expression in forms with mesifurane-rich fruit and barely detectable expression in forms lacking mesifurane in fruit. This result supports the key role of the FaOMT gene in mesifurane content variation in strawberry fruit (Zorrilla-Fontanesi et al., 2012).

In the strawberry collection analyzed, the functional (active) allele of the FaOMT gene (FaOMT+) was identified in 86.1% of forms out of 43 analyzed genotypes. The nonfunctional (inactive) allele (FaOMT–) was identified in 48.8% forms out of 43 analyzed genotypes. The combination of active and inactive alleles of the FaOMT gene (FaOMT+ FaOMT– genotype) was detected in 34.9% of the analyzed forms. The homozygous state of the active allele of the FaOMT gene (FaOMT+ FaOMT+ genotype) was identified in 51.2% of the analyzed forms, and the homozygous state of the inactive allele (FaOMT– FaOMT–), in 13.9%. An example of FaOMT allelic state analysis is shown in the Figure, a, and the overall results are summarized in Table 2.

Among the analyzed wild species of the genus Fragaria L., the FaOMT+ allele (FaOMT+ FaOMT+ genotype) was detected in F. orientalis Los., F. moschata Duch., and F. virginiana Duch. ssp. platypetala. It should be noted that the target products of the FaOMT-SI/NO marker are not amplified in French variety Capron Royale (F. moschata Duch.) with high fruit mesifurane content (Cruz-Rus et al., 2017). This result might be due to substitutions at the primer-binding site or the effect of other genetic factors on mesifurane content. It requires additional studies.

Among the 22 Russian strawberry varieties analyzed, the homozygous state of the functional FaOMT+ allele was identified in 59.1% of forms and the heterozygous combination, in 27.3%. The homozygous state of the nonfunctional FaOMT– allele was identified in 13.6% of Russian strawberry varieties. Among the analyzed 16 foreign strawberry varieties, 56.3% of forms had the FaOMT+ FaOMT– genotype, 37.5% forms had the FaOMT+ FaOMT+ genotype, and 6.2% forms had the FaOMT– FaOMT– genotype. The predominance of the heterozygous combination alleles of the FaOMT gene in foreign strawberry varieties is consistent with literature data (Cruz-Rus et al., 2017).

The γ-decalactone content in strawberry fruit is controlled by the FaFAD1 gene (candidate gene 24414), which is mapped to the distal region of the long arm of chromosome III-2 in the F. × ananassa Duch. genome (Sánchez-Sevilla et al., 2014). Comparison of the genomes of the Elyana variety (γ-decalactone is produced) and the Mara des Bois variety (γ-decalactone is not produced) shows that the γ-decalactone content in strawberry fruit is determined by the expression of one functional FaFAD1 allele, and the absence of γ-decalactone in fruit is caused either by mRNA FaFAD1 gene transcription block, or by the lack of the active allele from the genome (Chambers et al., 2014).

Primers FaFAD1-F/R amplify a 500 bp fragment at the 5’ end of gene 24414. This fragment is not amplified in genotypes with undetectable γ-decalactone in fruit (Chambers et al., 2014). The relationship between the presence of the functional FaFAD1 allele in the genome and the γ-decalactone content in fruit was tested on three hybrid combinations: Elyana (γ-decalactone is produced) × Mara des Bois (γ-decalactone is not produced), Elyana (γ-decalactone is produced) × 98 (γ-decalactone is not produced), and Mara des Bois (γ-decalactone is not produced) × 98 (γ-decalactone is produced). All strawberry genotypes with high γ-decalactone contents in the fruit possessed the functional FaFAD1 allele. The correlation between the presence of the functional FaFAD1 allele and γ-decalactone presence in the fruit was also confirmed by analysis of γ-decalactone-producing varieties Radiance, Albion, Winter Star, and Sweet Charlie and non-γ-decalactone-producing varieties Deutsch Evern, Strawberry Festival, LF9, and Mieze Schindler (Chambers et al., 2014). As reported in (Zorrilla-Fontanesi et al., 2012), in 93.3% of cases high γ-decalactone content in fruit is due to the presence of the FaFAD1 gene.

In the analyzed collection of 43 strawberry genotypes, the FaFAD1 gene was identified in 25.6% forms, including the wild species F. orientalis Los., F. moschata Duch., and F. ovalis Rydb. An example of FaFAD1 gene identification is shown in the Figure, b, and the results are shown in Table 2. The FaFAD1 gene is also present in the French variety Capron Royale (F. moschata Duch.) (Cruz-Rus et al., 2017). Among the 22 analyzed Russian strawberry varieties, the FaFAD1 gene was identified in 9.1% of forms (varieties Bylinnaya and Kupchikha). Among the analyzed 16 foreign strawberry varieties, FaFAD1 was identified in 37.5% forms. According to the data of Cruz-Rus et al. (2017), the FaFAD1 gene was identified in 40.0% of tested strawberry genotypes (F. × ananassa Duch.) of non-Russian breeding.

The wider distribution of the FaFAD1 gene in the germ plasm of non-Russian strawberry varieties is presumably explained by the genetic proximity of many varieties due to the widespread use of the same parental forms in breeding (Most of the non-Russian strawberry varieties created after 1960 were obtained by crosses of seven parental forms (Lei et al., 2002.), and one or more of these forms could be a donor of the functional allele of the FaFAD1 gene.

Strawberry fruit aroma is a complex multicomponent trait, whose manifestation is determined by the expression of many
Полиморфизм генотипов земляники коллекции ФНЦ им. И.В. Мичурина по генам FaOMT и FaFAD1

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Table 2. Allelic diversity of the FaOMT and FaFAD1 fruit flavor volatile genes in strawberry varieties and wild species (1, allele is present; 0, absent)

| No. | Genotype               | FaOMT  | FaFAD1 | FaOMT  | FaFAD1 |
|-----|------------------------|--------|--------|--------|--------|
|     |                        | 217 bp | 248 bp | 500 bp |        |
| 1   | *F. orientalis* Los.  | 0      | 1      | 1      |        |
| 2   | *F. moschata* Duch.  | 0      | 1      | 1      |        |
| 3   | *F. ovalis* Rydb.     | 1      | 0      | 1      |        |
| 4   | *F. virginiana* Duch. ssp. platypetala | 0 | 1 | 0 |        |
| 5   | Alyona                 | 0      | 1      | 0      |        |
| 6   | Bogema                 | 0      | 1      | 0      |        |
| 7   | Bylinnaya              | 1      | 0      | 1      |        |
| 8   | Vityaz                 | 1      | 1      | 0      |        |
| 9   | Girlyanda              | 0      | 1      | 1      |        |
| 10  | Divnaya                | 0      | 1      | 0      |        |
| 11  | Zenit                  | 0      | 1      | 1      |        |
| 12  | Karnaval               | 0      | 1      | 0      |        |
| 13  | Krymchanka 87          | 0      | 1      | 0      |        |
| 14  | Kupchikha              | 1      | 0      | 1      |        |
| 15  | Lastochka              | 0      | 1      | 0      |        |
| 16  | Neznakomka             | 1      | 1      | 0      |        |
| 17  | Olimpiyskaya nadezhda | 1      | 0      | 0      |        |
| 18  | Privilekatelnaya       | 1      | 1      | 0      |        |
| 19  | Rusich                 | 1      | 1      | 0      |        |
| 20  | Solovushka             | 1      | 1      | 0      |        |
| 21  | Studencheskaya         | 0      | 1      | 0      |        |
| 22  | Sudarushka             | 1      | 0      | 0      |        |

Electrophoresis profiles of markers (a) FaOMT-SI/NO and (b) FaFAD1 at strawberry genotypes.
Lanes: 1, Red Gauntlet; 2, Lastochka; 3, Torpeda; 4, Zenit; 5, Sonata; 6, Karmen; 7, Bylinnaya; 8, Samson; 9, Bogema; 10, Sudarushka; 11, Kupchikha; 12, *F. ovalis* Rydb.; 13, *F. virginiana* Duch. ssp. platypetala; 14, *F. moschata* Duch.; M, molecular weight ladder.
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genetic factors. In this regard, the most promising forms in breeding for fruit aroma are genotypes with several fruit flavor volatile genes in the genome. In the analyzed collection of strawberry genotypes, the combination of functional alleles of the FaOMT and FaFAD1 genes was detected in 16.3 % of the forms (see Table 2). Among them, the wild species F. orientalis Los., F. moschata Duch, and strawberry variety Red Gauntlet combine the functional allele of the FaFAD1 gene with the homozygous state of the active allele of the FaOMT gene. Foreign strawberry varieties Marshall, Sonata, Tokado, and Vima Tarda combined the functional allele of the FaFAD1 gene with the heterozygous state of the FaOMT gene. The combination the functional alleles of the FaFAD1 and FaOMT genes was not found in the analyzed Russian strawberry varieties.

Conclusion

Thus, according to the results of molecular analysis of the FaOMT allelic state, the promising sources of high mesifuran content in breeding for fruit aroma are Russian strawberry varieties Alyona, Bogema, Girlyanda, Divnya, Zenit, Karnavali, Krymchanka 87, Lastochka, Studencheshkaya, Torpeda, Festivalnaya, and Flora, and foreign strawberry varieties Barlidaun, Elianny, Karnem, Samson, and Vima Zanta, which are characterized by the homozygous state of the functional allele of the FaOMT gene (FaOMT+FaOMT+ genotype). The sources of high γ-decalactone content in fruit varieties Bylinnaya, Kupchikha, Gigantella Maxim, Marshall, Sonata, Tokado, and Vima Tarda, which are characterized by the presence of the active allele of the FaFAD1 gene. The wild species F. orientalis Los., F. moschata Duch., and strawberry variety Red Gauntlet, combining the functional allele of the FaFAD1 gene with the homozygous state of the active allele of the FaOMT gene, are complex sources of high mesifuran and γ-decalactone contents in fruit.

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