A AVALIAÇÃO DO POLIMORFISMO DE CULTIVARES DE FRAMBOESA RUSSOS UTILIZANDO MARCADORES DE MICROSATÉLITE

THE ASSESSMENT OF POLYMORPHISM OF RUSSIAN RASPBERRY CULTIVARS USING MICROSATELLITE MARKERS

ОЦЕНКА ПОЛИМОРФИЗМА РОССИЙСКИХ СОРТОВ МАЛИНЫ С ПОМОЩЬЮ МИКРОСАТЕЛЛИТНЫХ МАРКЕРОВ

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Received 12 June 2018; received in revised form 30 November 2018; accepted 01 December 2018

RESUMO

As bagas de framboesa têm excelentes qualidades nutricionais e gustativas e contêm altos níveis de compostos com atividade antioxidante (antocianinas e outros). Criação de framboesas para a melhoria das características dos alimentos pode ser significativamente acelerada através da aplicação de marcadores moleculares. Dezessete variedades de framboesa e amora-preta, incluindo treze cultivares russas com ampla variação da coloração das bagas, foram analisadas utilizando 13 locos de microssatélites. Foi mostrado que os locos FJ194454, FJ194451 e AF29236 são específicos apenas para framboesas, e o locus FJ194450 é apenas para amora-preta. Foi estabelecido que os locos microssatélites estudados podem ser utilizados para identificar cultivares de framboesa da seleção russa. Foi mostrado que variedades com diferentes colorações diferem no número de alelos em alguns locos microssatélites, o que pode ser usado para seleção assistida por marcadores para o conteúdo de antocianinas.

Palavras-chave: *framboesa, seleção assistida por marcadores, microssatélites, antocianinas.*

ABSTRACT

The raspberry berries have outstanding nutritional and taste qualities and contain high levels of compounds with antioxidant activity (anthocyanins and others). Breeding of raspberries for the improvement of food traits can be significantly accelerated through the application of molecular markers. Seventeen varieties of raspberry and blackberry, including thirteen Russian cultivars with a wide variation of the berries coloring, were analyzed using 13 microsatellite loci. It is shown that the loci FJ194454, FJ194451, and AF29236 are specific only for raspberry, and the locus FJ194450 is only for blackberry. It has been established that the studied microsatellite loci can be used to identify raspberry cultivars of the Russian selection. It is shown that varieties with different coloring differ in the number of alleles in some microsatellite loci, what can be used for marker-assisted selection for the content of anthocyanins.

Keywords: *raspberry, marker-assisted selection, microsatellites, anthocyanins.*

DOI: 10.52571/PTQ.SI1.2018.131_PeriodicoES.pdf

АННОТАЦИЯ

Ягоды малины обладают высокими пищевыми и вкусовыми качествами и содержат большое количество антоцианов – соединений с высокой антиоксидантной активностью. Селекция малины на улучшение пищевых свойств может быть значительно ускорена с помощью молекулярных маркеров. 17 сортов малины и ежевики, в том числе 13 – российской селекции с широкой вариабельностью окраски ягод, были проанализированы по 13 микросателлитным локусам. Показано, что локусы FJ194454, FJ194451 и AF29236 являются специфичными только для малины, а локус FJ194450 – только для ежевики. Установлено, что изученные микросателлитные локусы могут быть использованы для идентификации сортов малины российской селекции. Показано, что сорта с различной окраской различаются по числу аллелей в некоторых микросателлитных локусах, что может быть использовано для маркерной селекции на содержание антоцианов.

Ключевые слова: *малина, маркерная селекция, микросателлиты, антоцианы.*

INTRODUCTION

The berry crops of temperate climatic zones are very popular in the world due to their dessert taste and beneficial health properties. Compared with fruit trees, they have a number of advantages: early fruiting, are less demanding to warm and do not suffer from the periodicity of fruiting. Berries are especially valuable foodstuff because of the high content of anthocyanins with strong antioxidant activity, which it contains 4 times more than in fruits, 10 times more than in vegetables and 40 times more than in cereals (Halvorsen *et al.*, 2002). One of the most popular berry crops in Russia is raspberry. Russia takes the first place in the world in raspberry production – about 120-140 thousand tons annually (FAOSTAT, 2018; Bassil *et al.*, 2012).

In the last decades, the main direction in the fruit breeding was the improvement of agronomic traits. However, in recent years, for most breeding programs, the importance of fruit quality improvement for new cultivars has increased and now it is being considered along with yield and resistance to pathogens (Mezzetti *et al.*, 2018; Strautina *et al.*, 2012). New varieties with a high content of biologically active compounds will be able to help in protecting the human body from diseases. However, the classical breeding of berry crops is a long-term and expensive process, for example, the duration of breeding a new raspberry variety reaches 15 years (Graham and Jennings, 2009).

The use of marker-assisted selection can significantly speed up this process. Different DNA markers were used to identify Rubus varieties and assess the genetic relationships between them – RAPD (Wang *et al.*, 2010; Badjakov *et al.*, 2005), SSR (Castillo *et al.*, 2010), AFLP (Miyashita *et al.*, 2015; Moore and Hoashi-Erhardt, 2012), but generally few molecular markers associated with economically valuable traits are known for Rubus (Bushakra *et al.*, 2015; Graham *et al.*, 2004). There are studies on the development of molecular markers linked with resistance to viral diseases (Ward *et al.*, 2012; Umar *et al.*, 2010), primocane fruiting and thornless (Castro *et al.*, 2013), cane splitting (Woodhead *et al.*, 2013; Fernandez-Fernandez *et al.*, 2011). More recently, genes related to the biosynthesis of flavonoids were investigated in Rubus species (Rafique *et al.*, 2016; Gutierrez *et al.*, 2017) and this will contribute to the development of molecular markers associated with anthocyanin pathway, as they are very little known for raspberry (Bushakra *et al.*, 2013).

It is known that imported varieties do not fully correspond to local soil and climatic conditions, which lead to reduced productivity and are susceptible to biotic and abiotic stresses. Despite the great economic importance of raspberry, there is very few research on molecular markers in Russian cultivars: study for identification of a marker for primocane fruiting was carried out (Sobolev *et al.*, 2009). The aim of the work was to evaluate microsatellite markers on raspberry cultivars of the Russian breeding centers, determine the degree of their polymorphism and assess its possibility for identification of genotypes with different content of biologically active compounds as the first stage of marker-assisted selection for this valuable trait.

MATERIALS AND METHODS

Seventeen cultivars of Rubus species were used in this study. They included 13 Russian (Atlant, Babye Leto II, Bryanskoe Divo, Beglyanka, Gerakl, Zhelty Gigant, Zolotaya Osen, Ispolin, Oranzhevoe Chudo, Patriciya, Pingvin, Solnyshko, Tarusa) and two UK (Glen Ample, Joan J) raspberry cultivars, and two blackberry varieties – Brzezina (Poland) and Natchez (USA). Genomic DNA from raspberry leaves was isolated using the CTAB-method according to Nunes *et al.* (2011). Microsatellite loci (13 pieces) for raspberry varieties were selected from the list of fully sequenced berry plants loci in GenBank. Using the Primer3 software, the primers for each of the loci were selected (Table 1). For the PCR, 50 µl of the reaction mixture of the following composition were used: 2 µl of template DNA (50 ng/µl), 0.5 µl of primers (10 pM) each, 10 µl of Screen mix (Eurogene, Russia) and 37 µl of Milli-Q water. PCR was performed on DNA Amplifier T100 Thermal Cycler (Bio-Rad, USA).

The temperature mode of the reactions was selected for each pair of primers taking into account their nucleotide composition (Table 1). The PCR conditions are as follows: initial denaturation at 96°C for 3 min, then 32 cycles with the next mode: denaturation at 94°C – 30 s, annealing for 20 s, elongation – 40 s at 72°C, final elongation – 5 min at 72°C. Electrophoresis of the amplification products was carried out in a vertical 6.8% PAAG (1x TBE-buffer). After electrophoresis, the gels were stained in an aqueous solution of the ethidium bromide and visualized in UV light. As a standard, size markers O'GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, USA) were used.

RESULTS AND DISCUSSION:

Microsatellites (SSR – simple sequence repeat) are widely used for plant genotyping because they are highly informative, codominant, and multi-allele genetic markers (Vieira *et al.*, 2016). Due to these properties, they are an important way of comparative mapping and identification of genomic regions associated with economically valuable traits in cultivated species (Bushakra *et al.*, 2015; Patamsytė *et al.*, 2010). These markers were usefully used on various Rubus species – R.idaeus (Woodhead *et al.*, 2008), R. *occidentalis* (Dossett *et al.*, 2012), R.arcticus (Kostamo *et al.*, 2013), etc.

For the assessment of Russian raspberry cultivars and for comparing imported blackberry varieties, we used 13 loci, of which 9 loci were present in both species, and 4 were specific only for raspberry (FJ194454, FJ194451, AF29236) or blackberry (FJ194450). Such a distinction is very important for cultivar identification of berry plants. Here, our data partially corresponds to the results obtained by Castillo *et al.* (2010), where the FJ194450 locus was also absent in raspberry varieties. However, in this work, the FJ194454 and FJ194451 loci were present in tested blackberry varieties and their hybrids, whereas we did not find them in the DNA of the blackberry varieties Brzezina and Natchez (Figures 1 and 2).

Increasing the consumption of 'healthy compounds' berries, including vitamin C, polyphenols, anthocyanins, and other flavonoids, is an attractive strategy for improving human health (Diamanti *et al.*, 2012). Anthocyanins with powerful antioxidant activity are one of the most valuable components of the berries. For example, the high content of anthocyanins in black raspberries increases interest in breeding new cultivars (Bassil *et al.*, 2014). Since anthocyanins are pigments, their content correlates with coloring — for example, in raspberries, it varies from 2 mg/100 g in a variety with yellow berries to 325 mg/100 g in a variety with blackberries (Bobinaite *et al.*, 2012; Kerber *et al.*, 2018). In our work, we used raspberry cultivars with a wide range of berry color – from light yellow (Zolotaya Osen) to dark red (Pingvin) and this may be due to the wide variability in anthocyanin content. Genetic certification of raspberry and blackberry cultivars with the different coloring of berries showed a difference in the number of alleles for some microsatellite loci (Figure 3, 4). This may be associated with different alleles of the structural and regulatory genes of the anthocyanin biosynthesis.

CONCLUSIONS:

Thus, it was shown that microsatellite markers can be used genetic certification of various Russian raspberry cultivars as the first stage of marker-assisted selection for improving their nutritional traits. Further works will involve the biochemical analysis of differently colored berry cultivars and sequencing of allelic variants of genes linked with nutritional traits. These genes can act as molecular markers in the breeding programs for improving the nutritional value of raspberry cultivars.

ACKNOWLEDGMENTS:

The work was financially supported by the Ministry of Education and Science of the Russian Federation (project No. 14.574.21.0149, unique project identifier RFMEFI57417X0149).

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***Table 1**. Sequences of primers for microsatellite markers in raspberry and blackberry plants*

Locus	Primer sequences	Temperature, °C
FJ194452	F: CGACACCGATCAGAGCTAATTC R: ATAGTTGCATTGGCAGGCTTAT	62
FJ194453	F: GAAACAGGTGGAAAGAAACCTG R: CATTGTGCTTATGATGGTTTCG	59
FJ194454	F: ATTCAAGAGCTTAACTGTGGGC R: CAATATGCCATCCACAGAGAAA	52
FJ194455	F: AGCAACCACCACCTCAACTAAT R: CTAGCAGAATCACCTGAGGCTT	51
FJ194444	F: GGTTCGGATAGTTAATCCTCCC R: CCAACTGTTGTAAATGCAGGAA	51
FJ194445	F: CCATCTCCAATTCAGTTCTTCC R: AGCAGAATCGGTTCTTACAAGC	50
FJ194446	F: AAAGACAAGGCGTCCACAAC R: GGTTATGCTTTGATTAGGCTGG	56
FJ194447	F: CACCAATTGTACACCCAACAAC R: GATTGTGAGCTGGTGTTACCAA	54
FJ194448	F: CAGTCCCTTATAGGATCCAACG R: GAACTCCACCATCTCCTCGTAG	50
FJ194449	F: CGACAACGACAATTCTCACATT R: GTTATCAAGCGATCCTGCAGTT	53
FJ194450	F: CAACCTAATGACCAATGCAAGA R: GCAGAATCCATTCTCTTGTTGA	50
FJ194451	F: GGACACGGTTCTAACTATGGCT R: ATTGTCGCTCCAACGAAGATT	56
AF292369	F: TGTCCGATCCTTTTCTTTGG R: CGCTTCTTGATCCTTGACTTGT	55

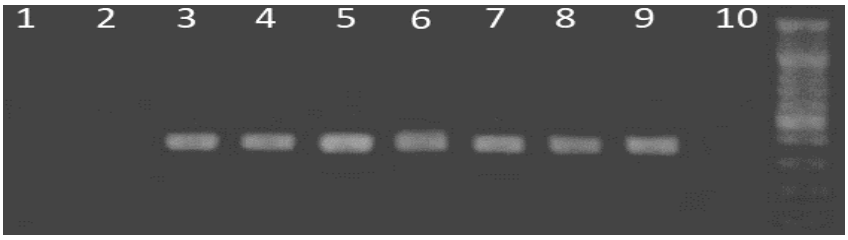

Figure 1*. PCR analysis for the presence of the locus FJ194451 (size 373 bp). 1 – Brzezina, 2 – Natchez, 3 – Atlant, 4 – Bryanskoe Divo, 5 – Zolotaya Osen, 6 – Ispolin, 7 – Oranzhevoe Chudo, 8 – Pingvin, 9 – Gerakl, 10 – water. 1% agarose gel, size marker - O'RangeRuler size 100 bp DNA Ladder (Thermo Fisher Scientific, USA).*

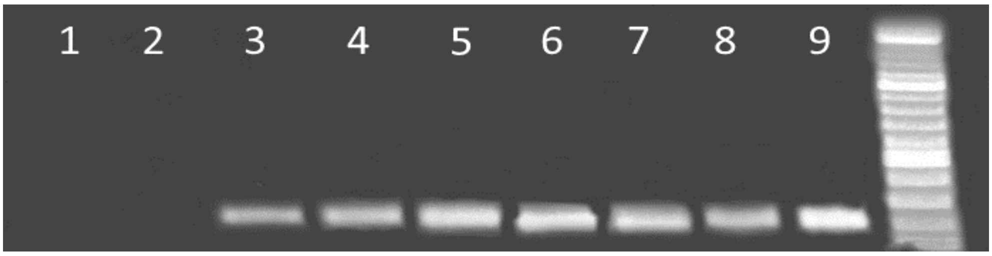

Figure 2*. PCR analysis for the presence of the locus FJ194454 (size 176 bp). 1 – Brzezina, 2 – Natchez, 3 – Atlant, 4 – Bryanskoe Divo, 5 – Zolotaya Osen, 6 – Ispolin, 7 – Oranzhevoe Chudo, 8 – Pingvin, 9 – Gerakl. 1% agarose gel, size marker – O'RangeRuler size 100 bp DNA Ladder (Thermo Fisher Scientific, USA).*

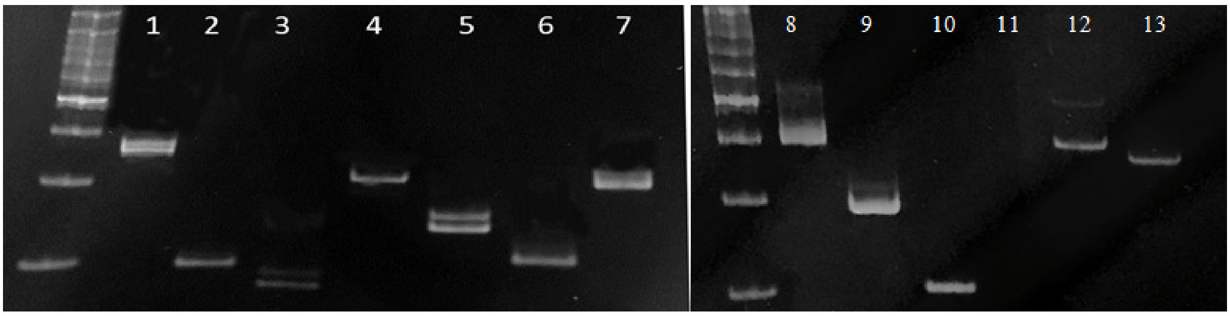

Figure 3*. Microsatellite loci of the raspberry cultivar Patriciya (bright purple berries). 1 – FJ194452, 2 – FJ194453, 3 – FJ194454, 4 – FJ194455, 5 – FJ194444, 6 – FJ194445, 7 – FJ194446, 8 – FJ194447, 9 – FJ194448, 10 – FJ194449, 11 – FJ194450, 12 – FJ194451, 13 – AF292369. Specific locus for blackberry FJ194450 is missing.*

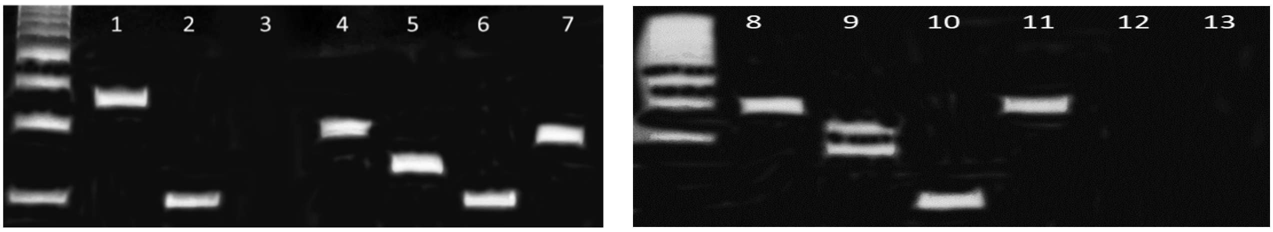

Figure 4*. Microsatellite loci of the blackberry cultivar Brzezina (black berries). 1 – FJ194452, 2 – FJ194453, 3 – FJ194454, 4 – FJ194455, 5 – FJ194444, 6 – FJ194445, 7 – FJ194446, 8 – FJ194447, 9 – FJ194448, 10 – FJ194449, 11 – FJ194450, 12 – FJ194451, 13 – AF292369. Specific loci for raspberry (bands 3, 12, 13) are missing.*