Assessment of the adaptive potential of strawberry genotypes based on the development of endophytic microbiota and identified R-genes

I V Luk’yanchuk, K V Zaitseva, A S Lyzhin

I.V. Michurin FSC, 30, Michurin St., Michurinsk, Tambov Region, 393774, Russia

E-mail: irina.lk2011@yandex.ru

Abstract. The paper presents the results of a study of the strawberry genotypes (Fragaria L.) by characteristics of endophytic microbiota and genetic determinants of resistance to fungal pathogens. Highly adaptive genotypes were identified: F. virginiana Duch. ssp. platypetala, F. orientalis Los., F. ovalis Rydb., F. moschata Duch., 298-19-9-43, Urozhaynaya CGL, Flora, Privlekatelnaya and Troitskaya. These strawberry forms are characterized by a high frequency of testing of bacterial microbiota, which suppresses endophytic fungal pathogens. The wild strawberry F. virginiana Duch. ssp. platypetala is characterized by red stele root rot resistance (Rpf1 gene). Strawberry varieties Borovitskaya and Elianny are characterized by anthracnose resistance (Rca2 gene).

1. Introduction

Horticulture is a risky and energy intensive industry, so its effectiveness is largely determined by stable yields and high quality products. Strawberry is an ecologically plastic crop, but it is very sensitive to growing conditions. In the conditions of climate destabilization, strawberry plants are exposed to multiple effects of a complex of stress factors, which lead to an increase in the tension of the plant energy balance and the decrease in their resistance potential, and, as a consequence, plant productivity.

Immunodeficiency of plants, caused by abiotic stress, led to the activation of the endophytic microbiota (fungi, bacteria and mixed associations), which provoked the spread of diseases of a known and unclear etiology (necrosis and deformation of leaves, premature shedding of leaves, the death of generative buds, shedding of flowers and ovaries, and death of plants) [1]. However, endophytic microorganisms can also have a positive effect on the growth and development of the host plant due to the production of biologically active substances (phytohormones, vitamins, antibiotics), improved transport of water and nutrients and participation in the regulation of osmotic pressure and nitrogen nutrition [2, 3]. The presence and ratio of the species composition of endophytic microbiota are an important diagnostic characteristic of the functional state of plants when assessing their adaptive potential. In this regard, the endophytic microbiota is an informative bioindicator of the functional state of plants.

An important characteristic of the strawberry adaptive potential is also their resistance to the most common exogenous fungal pathogens, the mass development of which can lead to a loss of up to 100% of the yield and the death of plants. Under these conditions, genetically determined ecological resistance of varieties and forms of plants becomes a necessary condition for economically feasible
cultivation of agricultural crops, and breeding is the most effective means of sustainable growth in the size and quality of yield of crops [4]. Identification of genotypes with a high level of adaptation to the weather and climatic conditions of the cultivation region and genetically determined resistance to the most important pathogens is a necessary condition for increasing the efficiency of the breeding and the horticultural industry.

The purpose of this study was to assess the adaptive potential of strawberry genotypes based on characteristics of endophytic microbiota and identified R-genes for identify promising forms.

2. Materials and methods
The studies were carried out in 2018-2020. The biological objects of the study were the strawberry genotypes (*Fragaria* L.) of different ecological and geographical origin.

The assessment of the adaptive potential of strawberry plants was carried out on the basis of characteristics of endophytic microbiota. Testing of the endophytic microbiota was carried out by a standard method by inoculating leaf explants in tubes on sterile agar culture medium. The explants were sterilized twice: immersed in alcohol and burned in the flame, after which they were placed with sterile tweezers on the beveled surface of the culture medium. Testing was carried out monthly in ten replicates. The testing period is May-October. The isolated microorganisms were cultured in tubes on a potato culture medium: potatoes – 300 g, agar-agar – 20 g, sterile water – 1000 ml. Sterilization mode is 1 atm. within 30 minutes. The result of testing plants for the presence of endophytic microbiota (bacteria, mixed microbiota, negative test) was expressed as a percentage of the total number of tests.

The fungal pathogens resistance genes of strawberry were identified by molecular genetic analysis using diagnostic DNA markers. The *Rpf1* red stele root rot resistance gene was identified with the dominant marker SCAR-R1A [5]. The *Rca2* anthracnose resistance gene was identified with the dominant SCAR marker STS-Rca2_240 [6]. 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 amplification was performed in T100 Thermal Cycler (Bio-Rad). PCR conditions for the PCR reactions were described by original publications [5, 6]. Amplification products were separated by the electrophoretic method in agarose gel (agarose concentration – 2%, running buffer – 1x TBE). Amplicon estimated sizes were performed using the Gene Ruler 100 bp DNA Ladder (Thermo Fisher Scientific).

3. Discussion of the results
Testing of strawberry varieties and forms for the presence of endophytic microbiota showed the exit to culture medium bacteria, and microbial associations, represented by fungi and bacteria with varying degrees of development of one or another agent (mixed infection). There were also tests in which nothing came out on the culture medium – a negative test for endophytic microbiota.

The isolated bacteria with different cultural traits belonged to the genus *Pseudomonas* (Figure 1).

Due to the fungicidal and fungistatic properties of toxins, the bacteria controls endophytic fungi, performing the protective function of the plant organism.

Mixed microbiota includes, along with bacteria, mainly dark-pigment fungi of the genus *Alternaria* and *Penicillium* (Figure 2).

The toxins of mixed microbiota are the most dangerous, since due to the antagonistic interaction (double induction) of endophytic fungi and bacteria, the stress load on the plant organism increases.

An important indicator of the state of a plant organism is a negative test for endophytic microbiota. It reflects the severity of paraneocrosis of the plant, turning into necrosis under adverse external influences. The accumulation of phenol oxidation products (quinones) leads to the death of both the endophytic microbiota and the cells of the host plant. The higher percentage of negative test for endophytic microbiota, reflecting the level of oxidative stress in a plant, the lower its adaptive capacity.
Since the endophytic microbiota is located inside the plant, its development is directly dependent on the physiological state of the host plant.

In connection with the above, it should be noted that plants with the greatest adaptation potential are characterized by a high frequency of testing of bacterial microbiota and the decrease of fungal and mixed microbiota, and the decrease of percentage of negative test for endophytic microbiota.

The high frequency of testing of bacterial microbiota (>90%) was noted in Russian strawberry varieties Urozhaynaya CGL, Flora, Privlekatelnaya and Troitskaya, hybrid 298-19-9-43, and wild species *F. virginiana* Duch. ssp. *platypetala*, *F. orientalis* Los., *F. ovalis* Rydb. and *F. moschata* Duch. (Figure 3).
Figure 3. Characteristics of endophytic microbiota in strawberry genotypes

At the same time, these strawberry forms were characterized by a lower level of mixed infection and negative tests for endophytic microbiota (<5%) compared with other studied genotypes, which indicate their higher adaptive potential.

An important characteristic of the adaptive potential of strawberry plants is also their resistance to exogenous fungal pathogens. Among the most dangerous strawberry diseases are anthracnose (*Colletotrichum acutatum*) and red stele root rot (*Phytophthora fragariae var. fragariae*).

In the analyzed collection of strawberry genotypes, the varieties Borovitskaya and Elianny are characterized by resistance to anthracnose (*Rca2* dominant gene). The wild species *F. virginiana* Duch. ssp. *platypetala* is characterized by resistance to red stele root rot (*Rpf1* dominant gene) (Table 1).

The molecular markers used for the analysis are dominant and do not allow revealing the allelic state of the studied *R* genes. In this regard, the *Rpf1* (*F. virginiana* Duch. ssp. *platypetala*) and *Rca2* (Borovitskaya, Elianny) genes can be in two states: heterozygous or dominant homozygous. However, earlier studies [7] showed that the selected form 933-4 from the hybrid combination *F. virginiana* Duch. ssp. *platypetala* × Rubinovyy kulon is characterized by a recessive homozygous state of the *Rpf1* gene, which indicates a heterozygous state of the *Rpf1* gene in the parental form *F. virginiana* Duch. ssp. *platypetala*.

Analysis of the origin of the strawberry variety Elianny did not allow us to clarify the allelic status of the *Rca2* gene, since there is no information on the presence or absence of the *Rca2* gene for the parental forms. The strawberry variety Borovitskaya was obtained in the hybrid combination Nadezhda × Red Gauntlet, in which the source of the *Rca2* gene is presumably the strawberry variety Nadezhda (*Rca2Rca2* or *Rca2rca2* genotype), since according to our data [8], the strawberry variety Red Gauntlet is characterized by a recessive homozygous state of the *Rca2* gene. In this regard, the strawberry variety Borovitskaya is presumably characterized by a heterozygous state of the *Rca2* gene (*Rca2rca2*).
Table 1. Allelic state of Rpf1 and Rca2 genes in strawberry genotypes according to the results of molecular genetic analysis

| Genotype                     | Rpf1 gene (marker SCAR-R1A) | Rca2 gene (marker STS-Rca2_240) | Putative genotype                  |
|------------------------------|------------------------------|----------------------------------|-----------------------------------|
| F. orientalis Los.           | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| F. moschata Duch.            | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| F. ovalis Rydb.              | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| F. virginiana Duch. ssp platypetala | 1                        | 0                                | Rpf1 rpf1 rca2 rca2               |
| 298-19-9-43                  | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Borovitskaya                 | 0                            | 1                                | rpf1 rpf1 rca2 rca2               |
| Krymchanka 87                | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Kubata                       | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Privlekatelnaya              | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Torpeda                      | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Troitskaya                   | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Urozhaynaya CGL              | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Feyerverk                    | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Flora                        | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Yuniol                       | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Elianny                      | 0                            | 1                                | rpf1 rpf1 rca2 R(r)ca2            |
| Gigantella                   | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Kimberly                     | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Korona                       | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Red Gauntlet                 | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Vima Tarda                   | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |
| Vima Zanta                   | 0                            | 0                                | rpf1 rpf1 rca2 rca2               |

4. Conclusion
Thus, as a result of the assessment of the characteristics of the endophytic microbiota, highly adaptive genotypes were identified: F. virginiana Duch. ssp. platypetala, F. orientalis Los., F. moschata Duch., 298-19-9-43, Urozhaynaya CGL, Flora, Privlekatelnaya and Troitskaya. The Rpf1 red stele root rot resistance gene was identified in the wild strawberry F. virginiana Duch. ssp. Platypetala. The Rca2 anthracnose resistance gene was identified in the strawberry varieties Borovitskaya and Elianny.

References
[1] Ishchenko L A, Kozaeva M I, Maslova M V and Zaitseva K V 2007 Horticulture and viticulture 4 2–4
[2] Smolyakova V M, Puzanova L A, Yakuba G V, Podgornaya M E and Chernezova S R 2008 Horticulture and viticulture 5 20–21
[3] Sakhibgareev A A and Menlikeev M Ya 2008 Vestnik of the Russian agricultural science 3 60–62
[4] Zhuchenko A A 2009 Bulletin of Orel State Agrarian University 3 8–12
[5] Haymes K M, Van de Weg W E, Arens P, Maas J L, Vosman B and Den Nijs A P M 2000 J. Amer. Soc. Hort. Sci. 125(3) 330–339. DOI: https://doi.org/10.21273/JASHS.125.3.330
[6] Lerceteau-Kohler E, Guerin G and Denoyes-Rothan B 2005 Theor. Appl. Genet. 111 862–870. DOI: https://doi.org/10.1007/s00122-005-0008-1
[7] Lyzhin A S, Luk’yanchuk I V and Zhbanova E V 2019 Proceedings on Applied Botany, Genetics and Breeding 180(1) 73–77. DOI: https://doi.org/10.30901/2227-8834-2019-1-73-77
[8] Lyzhin A S and Luk’yanchuk I V 2020 Agrarian Russia 12 30–34. DOI: https://doi.org/10.30906/1999-5636-2020-12-30-34