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Features of Seeds Microbiome for Spring Wheat Varieties from Different Regions of Eurasia

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***Abstract* — The aim of the study was to investigate the peculiarities of microbiome and seed bacterial endophytes isolated from different varieties of spring wheat grown in different areas of Eurasia (European part of Russia (Volga region), countries of Central Asia). The main method of the microbiome studies was PCR. Isolation of bacterial endophytes from the seeds allowed estimating their count and evaluating their activity towards *Fusarium oxysporum*. Quantitative analysis of the microbiome was performed on seeds from different spring wheat varieties (genotypes) obtained from the Russian Federation, the Republic of Kazakhstan and the Republic of Tadzhikistan. The differences in the bacterial endophytic microflora of the seeds and their activity towards *Fusarium oxysporum* were established. The seeds grown in Central Asia have higher count of both total microbiome and bacterial endophytes active against phytopathogens than the seeds grown in Volga region of the Russian Federation. Differences in the species composition of seed endophytic bacteria were established. Active strains of seed endophytic bacteria obtained from Tajikistan belonged to the species *Bacillus jeuvensis*. Active strains of bacterial endophytes were isolated from spring wheat seeds grown in Tadzhikistan for the development of highly potential biofungicides. The study results confirmed that seed microbiome depends on variety and the area of cultivation. The materials of the study can be useful for the evaluation of the seeds in the breeding of spring wheat and in the search for new biological agents for biofungicides development.**

Keywords* — *metagenomic analysis, bacterial endophytes, biological agents, biofungicides, Fusarium oxysporum, spring wheat, seeds

I. INTRODUCTION

Wheat is one of the major crops in the world. The growth of the world's population and global climate change dictate the need to develop methods to increase crop yields. The most important reasons for the low productivity of wheat are various stresses, including infectious diseases. Crop losses from wheat diseases in some years reach 30-40%. The chemical fungicides are mainly used to control diseases in the world and in the countries of the former Soviet Union. However, the development of pathogen resistance and the relatively high cost of chemical fungicides dictate the need to develop alternative disease control strategies. These strategies include the biological method of plant protection. However, the effectiveness of biological plant protection depends on the activity of the biological agent. The search, testing and application of new biological agents is one of the most important tasks in the development of integrated plant protection systems.

The Russian Federation, the Republic of Kazakhstan and the Republic of Tajikistan are among the regions of the world with developed production of wheat grain. Russia and Kazakhstan are among the major wheat exporters in the world. The Republic of Tajikistan is one of the oldest centers of wheat cultivation in Eurasia.

In the Volga region of Russia, as well as in Kazakhstan and Tajikistan, the main type of wheat is spring wheat *Triticum aestivum* L.

Among the most dangerous wheat fungal pathogens there are the Fusarium spp. The accumulation in the wheat grains the mycotoxins produced by these fungi is one of the most acute problems in the production of spring wheat grain both in the world and on the territory of the studied regions of Eurasia. The search of biological agents associated with wheat seeds for control diseases of this type is of particular importance.

For all three regions of Eurasia, in the cultivation of spring wheat, disease control occupies a special place. In this regard, the production of new biological agents for biological plant protection from natural sources, such as seeds, is an important task.

Development of wheat seeds is influenced by interaction of the genotype and environmental conditions, which can have a prolonged effect on the realization of potential characteristics of varieties taking into account variability and changeability of the main agroecological parameters. The analysis of the seeds properties is an integral part of genotype evaluation in wheat breeding programs because of high importance of these parameters in practice [4]. Interaction of plants and microorganisms is observed at all the stages of ontogenesis, determining the productivity of agricultural crops and their resistance to stress [5]. At present, plant microbiome studies attract researchers' attention [1, 2, 8, 14], including those that involve seeds [15]. Plant microbiome is a complex that consists of archaea, eubacteria, and fungi that inhabit plant (seed) surfaces [9] and endophytic microbiota [13]. The studies of microbiome and bacterial endophytes showed that they play an important role in the life of plants [3, 18], in particular, in protection from phytopathogens [12, 17]. The content and count of bacterial endophytes varied depending on the conditions, including the geographical area where they were isolated [11].

Bacterial endophytes are potential sources of biological agents for application in agriculture [6, 7, 16].

One of the most interesting aspects of studying endophytic bacteria is the assessment of the role of environmental factors and the plant genotype in the formation of their population, as well as activity against phytopathogenic fungi.

Quantitative studies of microbiome peculiarities of different varieties of wheat seeds are in the initial stage and this issue requires further investigation. The studies on bacterial endophytes of wheat seeds, including their biological activity towards pathogenic fungi, also draw researchers' attention. Studying this issue and obtaining new biological products based on endophytic bacteria can be the mechanisms for managing crop productivity in the context of global climate change.

II. MATERIALS AND METHODS

The study was conducted at the Department of General farming, crop protection and breeding at the Kazan State Agrarian University.

An object of the study: spring wheat (*Triticum aestivum L.*) seeds of the varieties grown in the Russian Federation (RF, variety Iolduz), the Republic of Kazakhstan (RK, variety Karagandinskaia 31) and the Republic of Tadzhikistan (RT, variety Sodokat).

The evaluation of the seed microbiome was performed by qPCR assay. DNA concentration of fungi and bacterial epiphytes in total DNA, isolated from the seeds by the conventional methods [10], was determined using primer pairs 5.8S-f 5'-CGC AGC AAA ATG CGA TAA GTA ATG TGA ATT G-3' and 5.8S-r 5'-CCC AAC ACC AAG CTG TGC TTG AG-3' for fungi and U1-f 5'-ACT CCT ACG GGA GGC AGC AGT-3' and U1-r 5'-GTA TTA CCG CGG CTG CTG GCA C-3'for bacteria. Reaction mixture of 25 µL comprised of 15 µL of qPCRmix-HS SYBR (Evrogen, Moscow, RF), 5 pM each primers and 5 µL of DNA with concentration 5 ng/µL. To build the standard curve dilution of DNA isolated from *Fusarium oxysporum,* ZUM2407 or *Pseudomonas putida* PCL1760 in plant DNA (5 ng/µL) were used.

Isolation of bacterial endophytes from the seeds was performed according to the method proposed by M. Simons, van der Bij A.J., Brand J., de Weger L.A., Wijffelman C.A., and Lugtenberg B.J.J. [19]. The microorganisms were cultivated in King B medium.

The evaluation of fungicide activity of the isolates towards *Fusarium oxysporum* ZUM2407 (the strain was provided by collection of the Kazan Federal University) was performed by confrontation test on Czapek's agar medium [20].

Identification of endophytic bacteria strains was carried out on the basis of a comparison of the sequences of the 16S rRNA gene fragment with those in the GenBank database. For the amplification of the fragments, we used universal primers 27fm (5'-AGA GTT TGA TCM TGG CTC AG -3) and R1522 (5'-AAG GAG GTG ATC CAG CCG CA-3') [21] and chromosomal DNA. The nucleotide sequence of the fragments was determined in the company Evrogen (Moscow, Russian Federation) using primers 27fm and R1522. The obtained chromatograms were analyzed using the software package Clont Manager 9 (Science and Education Software, USA). Nucleotide sequences were compared using the BLAST system

III. RESULTS AND DISCUSSION

Two varieties (RF and RT) were chosen for quantitative evaluation of the seeds microbiome. The results of the assays on micromycetes and bacteria total DNA are presented in Table 1.

The results of the quantitative evaluation showed that spring wheat seeds grown in Tadzhikistan were characterized by significantly higher DNA concentration of micromycetes and bacteria. Probably, warmer weather conditions in Central Asia contribute to more intensive colonization of seeds by microflora. While, in the conditions of European part of Russia, this process is less intensive.

In addition, a possible level of application of chemical fungicides may be a possible reason for such differences. If on wheat in the Republic of Tatarstan, fungicidal treatments were actively used, in Tajikistan these works were not carried out.

The results of isolation of bacterial endophytes from the varieties of spring wheat seeds are presented in Table 2.

TABLE I. TOTAL DNA OF MICROMYCETES AND BACTERIA OBTAINED FROM DIFFERENT VARIETIES OF SPRING WHEAT

Genotype	*Concentration of DNA of micromycetes, pg in 10 ng of total DNA*	*Concentration of DNA of bacteria, pg in 10 ng of total DNA*
RF	0.12±0.004	198.5±9.1
RT	0.62±0.029	1,315.2±58.1

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TABLE II. TOTAL COUNT OF BACTERIAL ENDOPHYTES AND NUMBER OF ACTIVE STRAINS (*FUSARIUM OXYSPORUM*) ISOLATED FROM DIFFERENT VARIETIES OF SPRING WHEAT, PCS, 2017.

Genotype	*CFU of bacterial endophytes, pcs per 100 kernels*	*Active isolates towards Fusarium oxysporum*
RF	3.0±0.11	0
RK	737.3±28.9	4
RT	636.2±30.4	29

Total count of bacterial endophytes, isolated from the seeds of spring wheat, was significantly higher in the varieties grown in Central Asia. When comparing the figures for the countries of Central Asia, it was found that the total number of endophytic bacteria in samples from Kazakhstan is higher, but the number of active strains is much lower than that of samples from Tajikistan. The difference in genotype parameters was more than by 200 times higher. However, only a minor part of the isolates from all the bacterial endophytes was active towards *Fusarium oxysporum*. The maximum number of isolates that inhibited the growth of phytopathogenic fungus was observed in the variety grown in Tadzhikistan. There were no strains active towards this fungus isolated from the varieties grown in Russia.

Possible differences in both the total number of endophytic bacteria and their activity with respect to the phytopathogenic fungus can be associated with climatic differences in the places where spring wheat is grown. In addition, the influence of the applied plant protection systems in each of the regions of Eurasia is possible.

The results of the evaluation of the antagonist activity of the isolates obtained from the seeds of different varieties of spring wheat are presented in Table 3.

Bacterial endophytes, isolated from the seeds of spring wheat variety grown in Kazakhstan, were characterized by low activity against *Fusarium oxysporum* and cannot be used as biological agents for biofungicides. A number of isolates from the variety of spring wheat grown in Tadzhikistan exerted high activity towards the phytopathogenic fungus.

TABLE III. THE AREA OF *FUSARIUM OXYSPORUM* COLONY GROWTH INHIBITION ON CZAPEK'S AGAR MEDIUM, MM, 2017.

Genotype	*Code of the isolate in the collection*	*Area of colony growth inhibition, mm*
RK	KGAU-2017-313, KGAU-2017-314, KGAU-2017-315, KGAU-2017-317	1.0±0.04
RT	KGAU-2017-332, KGAU-2017-333, KGAU-2017-335, KGAU-2017-336, KGAU-2017-337, KGAU-2017-340, KGAU-2017-343, KGAU-2017-344, KGAU-2017-346, KGAU-2017-352, KGAU-2017-355, KGAU-2017-356, KGAU-2017-357, KGAU-2017-358, KGAU-2017-359	2.0±0.06
	KGAU-2017-334, KGAU-2017-338, KGAU-2017-360	4.0±0.11
	KGAU-2017-361	6.0±0.14

The highest activity against *Fusarium oxysporum* was observed in one strain (Fig. 1). Further analysis showed that the all active isolates were a nonpathogenic form of *Bacillus* spp.

The species of endophytic bacteria of the genus Bacillus obtained from wheat seeds may be different. In addition, there are differences in the activity of these bacteria against phytopathogenic fungi. In the work of M. Pan et al. [22] *Bacillus megaterium* (BM1) and *Bacillus subtilis* (BS43, BSM0 y BSM2) were isolated from wheat seeds. They had a high antagonistic activity against *Fusarium graminearum*. S.V. Bacon and D.N. Hinton [22] studied the strain of the endophytic bacterium *Bacillus mojavensis* RRC 101 which showed high activity against *F. graminearum* and other *Fusarium* species.

The study of active endophytes of strains such as KGAU-2017-313, KGAU-2017-314, KGAU-2017-315, KGAU-2017-317 (RK) and KGAU-2017-332, KGAU-2017-333, KGAU-2017-335, KGAU-2017-336, KGAU-2017-337, KGAU-2017-340, KGAU-2017-343, KGAU-2017-344, KGAU-2017-346, KGAU-2017-352, KGAU-2017-355, KGAU-2017-356, KGAU-2017-357, KGAU-2017-358, KGAU-2017-359, KGAU-2017-334, KGAU-2017-338 (RT) showed that they all belong to the species *Bacillus subtilis*. At the same time, the strains KGAU-2017-360 and KGAU-2017-361 belonged to the species *Bacillus mojavensis*. Moreover, endophytic bacteria *Bacillus Bacillus mojavensis* were isolated only from wheat seeds from Tajikistan.

The differences found in the set of endophytic bacteria of the genus *Bacillus* depending on the region of wheat cultivation can be associated with both climatic factors and the system of crop management.

The studies showed that quantitative parameters for microbiome and bacterial endophytes, isolated from the seeds of spring wheat varieties grown in different geographical areas, differ significantly, which is in the good agreement with the data obtained by S. Klaedtke, M.A. Jacques, L. Raggi, A. Préveaux, S. Bonneau, V. Negri, V. Chable and M. Barret [11].

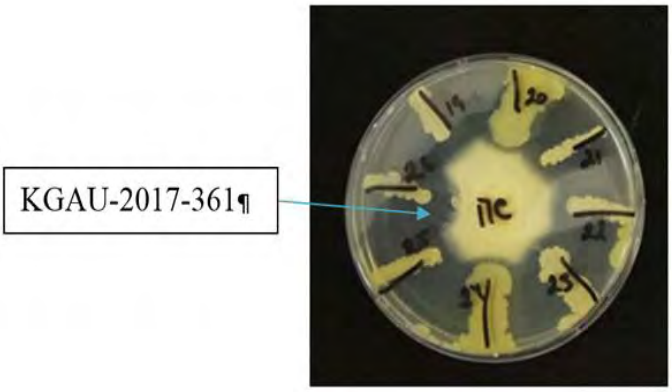

Fig. 1. The area of *Fusarium oxysporum* colony growth inhibition by bacterial endophytes isolated from the spring wheat grown in Tadzhikistan

In more southern countries (Tadzhikistan), microbiome and bacterial endophytes count was significantly different from that of more northern areas (Russia, Tatarstan). In warmer climate, development of bacterial endophytes is more intensive in wheat, and the number of isolates that demonstrate biological activity towards *Fusarium oxysporum* increases.

The revealed differences in the microbiome and in the population of endophytic bacteria of seeds of spring wheat from different regions of Eurasia can be associated not only with different climatic conditions, but also with different soil microbiomes, as well as with different crop management systems. In this regard, of particular interest is the study of the contribution of environmental factors, the system of crop management and genome of the plant. The solution of this problem will allow the use of transplantation techniques of valuable biological agents or whole microbiomes from some regions to others. Given that in the Volga region of Russia (Republic of Tatarstan), the level of use of chemical fungicides is significantly higher than in Kazakhstan and Tajikistan, and the activity of seed endophytes in relation to pathogens is extremely low, the possibility of such transfer and use of biological agents from Central Asian countries may be promising for integrated plant protection.

At the same time, in developing the technology of transferring biological agents from one region to another, it is necessary to take into account their ability to adapt to other environmental conditions, as well as their possible relationships with the aboriginal populations of endophytic bacteria.

To preserve the valuable native endophytic seed bacteria, it is necessary to carry out appropriate work on the collection of biological material in places with an ancient history of wheat cultivation and with minimal use of chemical plant protection products.

The results suggest that it is necessary to develop cooperation between different regions of Eurasia in studying the local set of microbiome seeds to develop effective protection systems for the sustainable development of wheat grain production.

IV. CONCLUSION

Qualitative analysis of microbiome and the study of bacterial endophytes microflora of the seeds can be used for evaluation of spring wheat varieties and ecological conditions of the area of cultivation. The results showed differences between the microbiomes and populations of endophytic bacteria of seeds of spring wheat from different regions of Eurasia. In a warmer climate, a more diverse population of endophytic bacteria is formed both quantitatively and in terms of activity against pathogens. Isolation of bacterial endophytes from the seeds of spring wheat is a potential source of biological agents for the development of new biofungicides.

An important advantage of the use of endophytic bacteria isolated from wheat seeds as biological agents for the creation of biological preparations for the protection of plants against diseases is their ecological safety.

The results can be the basis for developing search systems for promising biological agents to protect spring wheat from diseases in various regions of the world.

V. RECOMMENDATIONS.

Spring wheat variety Sadokat, grown in Tadzhikistan, is a valuable source of highly potential biological agents for biofungicides.

A promising biological agent for the creation of biological products for the protection of wheat is *Bacillus mojavensis* strains KGAU-2017-360 and KGAU-2017-361 obtained from seeds of spring wheat variety Sadokat.

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