IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-1, 2019, pp336-340
http://www.bipublication.com

Research Article

The Analysis of Microbiome and Bacterial Endophytes in Seeds during Evaluation of Spring Wheat Varieties (*TRITICUM AESTIVUM* L.)

[1]Radik I. Safin, [2]Shamil Z. Validov, [3]Liliia Z. Karimova,

[4]Dzhaudat I. Faizrakhmanov, [5]Rustam M. Nizamov

and [6]Airat R. Valiev

[1]Doctor of Agricultural Sciences, Professor, Deputy Head of the Department of General farming, crop protection and breeding Email: radiksaf2@mail.ru , ResearcherID: G-1594-2017
[2]PhD, Senior Research Scientist, Email: SZValidov@kpfu.ru, ResearcherID: D-2399-2014
[3]Candidate of Agricultural Sciences, Associate Professor at the Department of General farming, crop protection and breeding, Email: karimova-lcd@mail.ru, ResearcherID: E-9477-2014;
[4]Doctor of Economics, Professor, Rector, Email: info@kazgau.com
[5]Candidate of Agricultural Sciences, Associate Professor at the Department of land utilization and land cadaster Email: nizamovr@mail.ru, Tel.: +7-917-233-28-33, ResearcherID: F-9403-2017
[6]Candidate of Technical Sciences, Associate Professor at the Department of machinery operation and maintenance, Pro-rector of Scientific and International Affairs
Email: ayratvaliev@mail.ru, ResearcherID: F-9448-2017.

Kazan State Agrarian University
420008, 65 K. Marks Str., Kazan, Republic of Tatarstan, Russian Federation

[Received: 15/12/2018; Accepted: 22/01/2019; Published: 24/01/2019]

ABSTRACT.

Background. The rationale for the present study is the investigation of the peculiarities in the interaction between cultivated plants and microorganisms for yield increase and search for highly potential biological agents for the development of biopesticides. 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 (European part of Russia, countries of Central Asia). The main method of the microbiome studies was PCR that allowed for quantitative metagenomics analysis of wheat seeds colonization with bacteria and micromycetes. Isolation of bacterial endophytes from the seeds allowed the researchers to estimate their count and evaluate their activity towards *Fusarium oxysporum*.

Scope. Quantitative analysis of the microbiome of different spring wheat seed varieties (genotypes) was performed in the Russian Federation, Republic of Kazakhstan and Republic of Tadzhikistan. The differences by the parameters of bacterial and fungal DNA concentration in seeds of different varieties grown in Russia and Tadzhikistan were identified. The differences in the bacterial endophytic microflora of the seeds and their activity towards *Fusarium oxysporum*, depending on the area of cultivation, 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 the European part of Russia. Active strains of bacterial endophytes were isolated from spring wheat seeds grown in Tadzhikistan for the development of highly potential **biofungicides**.

Conclusion. The study results confirmed the dependence of the seed microbiome on the varietal peculiarities and the area of cultivation. The identified consistency in the increase of bacterial endophytes count in spring wheat grown in further southern regions (Tadzhikistan), as compared to northern regions (Russia, Tatarstan), can be used as a basis for further studies on the consistency of seed microflora development under different ecological conditions. 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.

Key words: PCR, metagenomic analysis, bacterial endophytes, biological agents, biofungicides, *Fusarium oxysporum*, spring wheat, seeds.

INTRODUCTION

Development of wheat seeds in influenced by the close interaction of the genotype and environmental conditions, which can exert a prolonged effect on the realization of potential characteristics of a certain variety 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 (Diekmann, 1996). Interaction of plants and microorganisms is observed at all the stages of ontogenesis, determining the productivity of agricultural crops and their resistance to stress (Grover et al., 2011). At present, plant microbiome studies attract researchers' attention (Bulgarelli et al., 2013; Haney , Ausubel, 2015; Mueller, Sachs, 2015; Berg et al., 2018), including those that involve seeds (Nelson, 2018). Microbiome is a complex aggregate that consists of archaea, bacteria, and micromycetes that inhabit epiphytic microbiota, including the one of the seeds (Hardoim et al., 2015), and endophytic microbiota (Malfanova et al., 2015).

The studies of microbiome and bacterial endophytes shoed that they play an important role in the life of plants (Santoyo et al., 2016; Calvo et al., 2017), in particular, in protection from phytopathogens (Ryan et al., 2008; Links et al., 2014). The content and count of bacterial endophytes varied depending on the conditions, including the geographical area where they were isolated (Klaedtke et al., 2016).

Bacterial endophytes are potential sources of biological agents for application in agriculture (Hallmann et al., 1997; Haggag, 2010; Orozco-Mosquedaa et al., 2018).

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.

MATERIALS AND METHODS.

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

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

The evaluation of the seeds microbiome was performed by PCR assay of the DNA concentration of micromycetes and bacterial epiphytes in total DNA, isolated from the seeds by the conventional methods (Kang et all., 1998).

Isolation of bacterial endophytes from the seeds was performed according to the method proposed by M. Simons et al. (1996). The microorganisms were cultivated in King B medium (King et al., 1954).

The evaluation of fungicide activity of the isolates towards *Fusarium oxysporum* (the strain was provided from the collection of the Kazan Federal University) was performed by the lysed zone (Validov et al, 2007). The phytophatogen was grown on Czapek's agar medium.

RESULTS.

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.

Table 1. 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

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

Table 2. 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. 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.
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.

Table 3. 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-2017-344,0 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

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. The highest activity against *Fusarium oxysporum* was observed in one strain (Figure 1). Further analysis showed that the isolate KGAU-2017-361 was a nonpathogenic form of *Bacillus spp*.

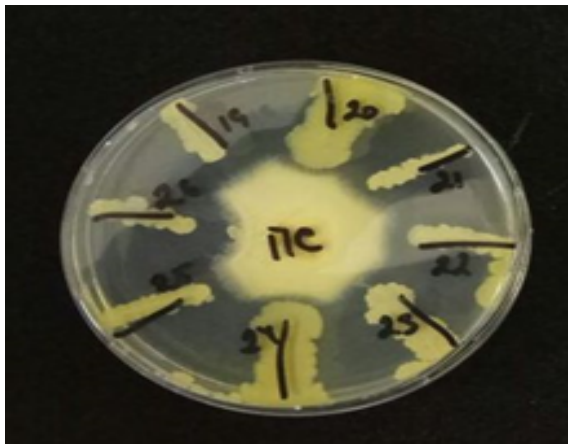

Figure 1. The area of *Fusarium oxysporum* colony growth inhibition by bacterial endophytes isolated from the spring wheat grown in Tadzhikistan

DISCUSSION

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 confirmed by the data obtained by S. Klaedtke et al., (2016). In more southern countries (Tadzhikistan), microbiome and bacterial endophytes count was significantly different than in more northern areas (Russia, Tatarstan). Probably, in warmer weather conditions, bacterial endophytes microflora development in wheat is more intensive, and the number of isolates that exert biological activity towards *Fusarium oxysporum* increases.

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. Isolation of bacterial endophytes from the seeds of spring wheat is a potential source of biological agents for the development of new biofungicides.

Recommendations.

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

ACKNOWLEDGEMENTS.

The study was conducted with the financial support provided by the Ministry of Education and Science of the Russian Federation. The subsidy agreement number is №14.610.21.0017. The unique identifier of the project is RFMEFI61017X0017.

REFERENCES.

1. Berg G., Rybakova D., Grube M., Köberl M., (2018). The plant microbiome explored: implications for experimental botany. J. Exp. Bot. 67, 995–1002.
2. Bulgarelli D., Schlaeppi K., Spaepen S., Ver Loren van Themaat E., Schulze-Lefert P. (2013) Structure and functions of the bacterial microbiota of plants Annu. Rev. Plant Biol., 64, pp. 807-838.
3. Calvo P., Watts D.B., Kloepper J.W., Torbert H.A. (2017) Effect of microbial-based inoculants on nutrient concentrations and early root morphology of corn (Zea mays). J. Plant Nutrit. Soil Sci., 180, pp. 56-70.
4. Diekmann M. (1996) Diseases in seed production. Eds. Seed Science and Technology. Proc. Train-the-Trainers Workshop Sponsored by Med-campus Programme (EEC), 24Apr.-9 May 1993, Amman. Aleppo, Syria, ICARDA.– P.170-175.
5. Grover M., Ali S. Z., Sandhya V., Rasul, A., Venkateswarlu, B. (2010): Role of microorganisms in adaptation of agriculture crops to abiotic stres. World J. Microbiol. Biotechnol. 27(5):1231-1240.
6. Haggag W. M. (2010) Role of entophytic microorganisms in biocontrol of plant diseases. Life Science Journal. 7(2):57-62.
7. Hallmann, J., Quadt-Hallmann A., Mahaffee W. F. and Kloepper J. W. (1997).Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43:895– 914.
8. Haney C.H., Ausubel F.M. (2015) Plant microbiome blueprints Science, 349, pp.788-789
9. Hardoim P. R., van Overbeek L. S., Berg G., Pirttilä A. M., Compant S., Campisano A., et al. (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 79:293–320.
10. Kang H.W., Cho Y.G., Yoon U.H. and Eun M.Y. (1998). A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. Plant Mol. Biol. Rep. 16: 1-9.
11. Klaedtke S et al (2016) Terroir is a key driver of seed-associated microbial assemblages. Environ Microbiol 18:1792–1804
12. Links M.G., Demeke T., Gräfenhan T., Hill J. E., Hemmingsen S. M. and Dumonceaux T. J. (2014). Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between

microorganisms within a shared piphytic microbiome on Triticum and Brassica seeds. New Phytol. 202(2): 542–553.

13. Malfanova N, Lugtenberg BJJ, Berg G (2013) Bacterial endophytes: who and where, and what are they doing there? In: Molecular microbial ecology of the rhizosphere. John Wiley & Sons, Inc., pp 391–403.
14. Mueller U.G. , Sachs J.L. (2015) Engineering microbiomes to improve plant and animal health. Trends Microbiol., 23 , pp. 606-617
15. Nelson E. B. (2018) The seed microbiome: Origins, interactions, and impacts. Plant Soil 422:7–34.
16. Orozco-Mosquedaa Ma del Carmen, Ma del Carmen Rocha-Granadosb , Glickc B. R., Santoyo G. (2018)Microbiome engineering to improve biocontrol and plant growth-promoting mechanisms. Microbiol. Res., 208 , pp. 25-31.
17. Ryan R.P. , Germaine K., Franks A., Ryan D.J. , Dowling D.N. (2008) Bacterial endophytes: recent developments and applications FEMS Microbiol. Lett., 278 , pp. 1-9.
18. Santoyo G., Moreno-Hagelsieb G, Orozco-Mosqueda Ma del. C, Glick B.R. (2016)Plant growth-promoting bacterial endophytes Microbiol. Res., 183 , pp. 92-99
19. Simons M., van der Bij A.J., Brand J., de Weger L.A., Wijffelman C.A., and Lugtenberg B.J.J. (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting Pseudomonas bacteria. Mol. Plant-Microbe Interact. 9: 600-607.Turner, T. R., James, E. K., and Poole, P. S. (2013). The plant microbiome. Genome Biol. 14:209. doi: 10.1186/gb-2013-14-6-209
20. Validov S., Kamilova F., Qi S., Stephan S., Wang J.J., Makarova N., Lugtenberg B. (2007) Selection of bacteria able to control *Fusarium oxysporum* f.sp. *radices-lycopersici* in stonewool substrate. J. Appl. Microbiol. 102:461–471.