

REVIEW

of the official reviewer for dissertation work of Rakhmetullina Aizhan Kazievna on the theme «Characteristics of miRNAs binding with mRNAs of transcription factor genes of agricultural plants» presented for the degree of Doctor of Philosophy (PhD) in the specialty «6D070100 - Biotechnology».

№	Criteria	Eligibility (one of the options must be checked)	Justification of the position of the official reviewer
1.	The topic of the thesis (as of the date of its approval) corresponds to the directions of development of science and/or state programs	1.1 Compliance with priority areas of science development or government programs:	<p>Corresponds to the priority area:</p> <p>Life Sciences and Health</p> <p>5.1 Biotechnology in agriculture and environmental protection</p> <p>5.1.1 Genomic editing technology, marker-related and genomic selection to improve economically valuable traits of plants and animals: Study plant miRNAs as key regulators of a variety of plant developmental and physiological processes, including genome integrity maintenance, development of root, stem, leaf, and flower organ, metabolism, and adaptive responses toward environmental stresses.</p> <p>5.1.8 Information technology in biology, agriculture and ecology: The advantages of conducting computational methods for identifying miRNAs in plants are as follows rapidness, lower expenses, and relatively easy comparison with experimental procedures.</p> <p>5.2 Biotechnology in medicine:</p> <p>5.2.2 Molecular, genomic, cellular and bioinformation technologies for the development of applied biology of personalized medicine: For humans, plant and animal miRNAs are exogenous (ex-miRNA or xe-miRNA) and do not have distinctive features among themselves. Therefore, they will be considered the general variety of endogenous miRNAs source for a human body, which means that all human genes can be their potential targets. The effect of ex-miR on human target genes and the consequences of this effect will depend on their concentration and the duration of their presence in the cells. Many studies have shown that miRNAs that enter the gastrointestinal tract with food (dietary miRNA) are used to regulate genes involved in</p>

			<p>various diseases.</p> <p>5.3 Development of domestic pharmaceutical science and industrial biotechnology.</p> <p>5.3.2 Technologies for obtaining valuable components from plant, animal and mineral raw materials using biotechnological methods: The miRNAs (biologically active substances) identified in the dissertation, which can regulate the expression of human genes, can be used as a means of therapy for humans and animals.</p>
		<p>1) The thesis was completed within the framework of a project or target program financed from the state budget (indicate the name and number of the project or program)</p> <p>2) The thesis was completed within the framework of another state program (indicate the name of the program)</p> <p>3) The dissertation corresponds to the priority direction of the development of science, approved by the Higher Scientific and Technical Commission under the Government of the Republic of Kazakhstan (indicate the direction)</p>	<p>The thesis is performed within project "Development of test-systems for early diagnosis of cardiovascular, oncological and neurodegenerative diseases based on miRNA associations and their target genes" № AP05132460 of the Ministry of education and science of the Republic of Kazakhstan.</p>
2.	Importance for science	<p>The work makes/does not make a significant contribution to science, and its importance is well disclosed/not disclosed</p>	<p>The dissertation work makes a significant contribution to the field of biotechnology and biology. The importance of the material given in the dissertation is well disclosed. This is confirmed by the analysis of local and foreign literature carried out by the doctoral student, as well as the identification of the theoretical and practical significance of the work. The identified plant miRNAs can affect human genes and can be used as biologically active compounds, since they easily penetrate the human body. All the main conclusions are based on experimental data and are reasonably well substantiated.</p>
3.	The principle of independence	<p>Self-reliance level:</p> <p>1) High;</p> <p>2) Medium;</p> <p>3) Low;</p>	<p>The level of independence of work is high, which is confirmed by the sufficient substantiation of scientific provisions. The principle of independence based on the results of research, the volume of publications in which the author of the thesis took a large part, the</p>

		4) No independence	obtained copyright certificate No. 15600 dated March 2, 2021 for the MirTarSeq program, it can be concluded that the author of the thesis is highly independent.
4.	The principle of inner unity	4.1 Justification of the relevance of the thesis: 1) Justified ; 2) Partially justified; 3) Not justified.	The topic of the dissertation is relevant and sufficiently justified since According to the Food and Agriculture Organization of the United Nations (FAO), the most used grain crops in the world are rice, corn and wheat. The growing consumption of these products requires an increase in their productivity.
		4.2 The content of the thesis reflects the topic of the thesis: 1) Reflects ; 2) Partially reflects; 3) Does not reflect	The content of the dissertation fully reflects the topic of the research, since it includes the study of the characteristics of the interaction of miRNA with mRNA of TF genes of wheat, maize and rice as the most used crops in human nutrition.
		4.3. The purpose and objectives correspond to the topic of the thesis: 1) correspond ; 2) partially correspond; 3) do not correspond	In the dissertation work, the author clearly formulates the goals and objectives of the research, which fully correspond to the topic of the dissertation. The number and sequence of tasks ensure the achievement of the research goal, namely the creation of databases of TF genes of the TCP, HSF, MYB, GRAS, ERF, C2H2 families and miRNA of <i>A. thaliana</i> , <i>O. sativa</i> , <i>Z. mays</i> and <i>T. aestivum</i> . Determination of the characteristics of interaction miRNA with mRNA of the TCP, HSF, MYB, GRAS, ERF, C2H2 TF genes of <i>A. thaliana</i> , <i>O. sativa</i> , <i>Z. mays</i> and <i>T. aestivum</i> . Establishing the characteristics of interaction rice, maize and wheat miRNAs with the mRNA of human genes.
		4.4 All sections and provisions of the thesis are logically interconnected: 1) completely interconnected ; 2) the interconnection is partial; 3) there is no interconnection	All sections and provisions of the thesis are logically interconnected. A literature review revealed and clearly substantiated the goals and objectives of the dissertation. The solution of the assigned tasks is logical and interrelated. The information required for presentation in each section is disclosed in detail. This presentation of the dissertation material allows more clearly perceiving the essence of the work. The results obtained logically serve as the basis for the conclusions and provisions submitted to the defense.
		4.5 The new solutions (principles, methods)	On the basis of a critical analysis of previously known solutions to

		<p>proposed by the author are reasoned and evaluated in comparison with the known solutions:</p> <ol style="list-style-type: none"> 1) there is a critical analysis; 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors 	<p>the research problem, the doctoral student proposes new solutions that she logically assessed and successfully used to obtain new original results. In most cases, the involvement of miRNAs in various biological processes has been described, but it has not been established which genes are the targets for these miRNAs. It is essential to solving this problem since it is necessary to know which miRNAs interact with target genes to carry out genetic-engineering manipulations.</p>
5.	Scientific novelty principle	<p>5.1 Are the scientific results and provisions new?</p> <ol style="list-style-type: none"> 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new) 	<p>The scientific results and provisions of the dissertation are completely new, which is confirmed by the presence of 3 articles in international journals included in the Web of Science and Scopus databases. In the dissertation work, in the format available for the MirTarget program, the following databases were created: genes of the TCP, HSF, MYB, GRAS, ERF, C2H2 and miRNA families of <i>A. thaliana</i>, <i>O. sativa</i>, <i>Z. mays</i>, and <i>T. aestivum</i>. The characteristics of miRNA interaction with mRNAs were established: the beginning of the miRNA binding site, the position of the site, and binding energy of miRNA with mRNA. Based on the data obtained using the MirTarget program, the author identified the miRNAs that bind to the largest number of mRNAs and described their characteristics.</p>
		<p>5.2 Are the dissertation findings new?</p> <ol style="list-style-type: none"> 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new) 	<p>The results and conclusions of the dissertation are based on a large experimental and theoretical material. The conclusions and obtained scientific results represent a completely new material, which eventually has been proved by a detailed analysis of the experimental data using the MirTarget program.</p>
		<p>5.3 Technical, technological, economic or management decisions are new and reasonable:</p> <ol style="list-style-type: none"> 1) completely new; 2) partially new (25-75% are new); 3) not new (less than 25% are new) 	<p>The dissertation work involved modern technical (bioinformatics) methods to obtain original results in order to reduce economic costs and simplify management decisions.</p>
6.	The validity of the main findings	<p>All main conclusions are based/are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of</p>	<p>All the main conclusions are based on strong evidence presented in the dissertation work. From a scientific point of view, the evidence presented using computer-based methods is quite convincing, since it</p>

		training in the arts and humanities)	was obtained by adequate bioinformatics methods.
7.	The main provisions for the defense	<p>It is necessary to answer the following questions for each provision separately:</p> <p>7.1 Is the provision proven?</p> <p>1) proven;</p> <p>2) rather proven;</p> <p>3) rather not proven;</p> <p>4) not proven</p>	<p>The main provisions for the defense have been proven:</p> <p>1. It has been shown that some genes out of the total number of 2403 genes of TCP, HSF, MYB, GRAS, ERF, C2H2 TF of <i>A. thaliana</i>, <i>O. sativa</i>, <i>Z. mays</i> and <i>T. aestivum</i> families can be miRNA targets of these organisms: This provision is proved by the presented results on the quantitative characteristics (the beginning of the miRNA binding sites in mRNA; the position of these sites in the 5'-untranslated region, in the protein coding sequence, and in the 3'-untranslated region of mRNA; free energy of miRNA interaction with mRNA (ΔG, kJ/mol); nucleotide interaction schemes between miRNA and mRNA) of the interaction of miRNA with mRNA of genes of each family of transcription factors TCP, HSF, MYB, GRAS, ERF, C2H2 <i>A. thaliana</i>, <i>O. sativa</i>, <i>Z. mays</i> and <i>T. aestivum</i>.</p> <p>2. The results of <i>in silico</i> studies of the influence of plant miRNAs on mRNA of TF genes of TCP, HSF, MYB, GRAS, ERF, C2H2 families of <i>A. thaliana</i>, <i>O. sativa</i>, <i>Z. mays</i> and <i>T. aestivum</i> showed that the many studied miRNAs can influence plant growth and development: This position is proved by the results of studying the effect of 1616 miRNAs on mRNA of 2403 genes of transcription factors. For each of the studied families of transcription factors, associations of miRNAs and target genes were identified, which can affect the growth and development of these plants.</p> <p>3. The miRNA binding sites in the mRNA of the studied genes of TCP, HSF, MYB, GRAS, ERF, C2H2 TF are located in the 5'UTR, CDS, and 3'UTR: This provision is convincing, for the first time it proves the presence of binding sites in all three regions (5'UTR, CDS, and 3'UTR) of mRNA target genes.</p> <p>4. The provision "Different quantitative characteristics of the interaction between miRNA and mRNA of the studied TF genes of the TCP, HSF, MYB, GRAS, ERF, C2H2 families indicate the effectiveness of the influence of miRNA on a number of genes of these TF families" was proved by using a set of quantitative</p>

			<p>characteristics of the interaction of miRNA and TF mRNA, which show the efficiency of the interaction of these molecules.</p> <p>5. Most of the studied miRNAs of <i>O. sativa</i>, <i>T. aestivum</i>, <i>Z. mays</i>, which are actively involved in plant growth and development can affect mRNA translation of human genes: This provision was formulated on the example of the results of interaction of 538 miRNAs with mRNA 2005 of human target genes.</p>
		<p>7.2 Is it trivial? 1) yes; 2) no</p>	<p>The provisions set forth previously unknown information about the interaction of miRNA with mRNA of plant genes. They are original and expand our understanding of the relationship of miRNA with the physiological functions of plants.</p>
		<p>7.3 Is it new? 1) yes; 2) no</p>	<p>The provisions are novel because they are based on original results obtained for the first time.</p>
		<p>7.4 Application level: 1) narrow; 2) medium; 3) wide</p>	<p>Defense provisions cover a wide range of key plant physiological properties and can therefore be used to increase plant productivity. The revealed dependences of the effect of miRNAs on human genes make it possible to use these miRNAs in the regulation of the expression of human genes involved in various diseases.</p>
		<p>7.5 Is it proven in the article? 1) yes; 2) no</p>	<p>According to the results of the dissertation research for 2018-2021, 17 printed works were published, including 3 articles in the international journal with the Impact Factor quoted in Scopus; 3 articles from the list of the Committee for Quality Assurance in Education and Science; 11 abstracts in materials of international conferences. Upon publication of the results, the reliability and significance of obtained results were proved.</p>
8.	<p>The principle of reliability Reliability of sources and information provided</p>	<p>8.1 Choice of methodology - is justified or the methodology is described in sufficient detail 1) yes; 2) no</p>	<p>Chapter 2 of the thesis describes in detail the methodology for conducting the experiment, and also outlines the rationale for its choice. In particular, in order to substantiate the choice of the MirTarget program, avtop compared the known programs for searching for miRNA binding sites with mRNA. To compare and demonstrate the effectiveness of MirTarget program over other programs, avtop used TAPIR</p>

		(http://bioinformatics.psb.ugent.be/webtools/tapir), psRNATarget (http://plantgrn.noble.org/psRNATarget/), and RNA 22 (https://cm.jefferson.edu/rna22/Interactive/). The following examples of search for binding sites by the MirTarget program and other programs show the great advantage of the MirTarget program
	8.2 The results of the thesis were obtained using modern methods of scientific research and methods of processing and interpreting data using computer technologies: 1) yes ; 2) no	The results of the dissertation work were obtained using modern methods of scientific research and methods of processing and interpreting data using computer technologies. As a result, author has carried out numerous bioinformatics studies that have reduced material costs comparatively thousands of times and significantly reduced the time spent on obtaining important information about the interaction of miRNA with target genes. Methods of computer analysis and search for miRNA binding sites in mRNAs of genes based on modeling hydrogen bonds using the MirTarget program.
	8.3 Theoretical conclusions, models, identified relationships and patterns have been proven and confirmed by experimental research (for areas of training in pedagogical sciences, the results have been proven on the basis of a pedagogical experiment): 1) yes ; 2) no	Theoretical conclusions, models, identified relationships and patterns have been proven and confirmed by a sufficient number of computational experiments. Experimental work is presented in several stages: in the format available for the MirTarget program, the following databases were created: for genes of the TCP, HSF, MYB, GRAS, ERF, C2H2 TF families, consisting of 442 genes of <i>A. thaliana</i> , 474 genes of <i>O. sativa</i> , 653 genes of <i>Z. mays</i> and 834 genes of <i>T. aestivum</i> ; for 428, 738, 325, and 125 miRNAs of <i>A. thaliana</i> , <i>O. sativa</i> , <i>Z. mays</i> , and <i>T. aestivum</i> . To predict binding sites with the MirTarget program, separate text files with their nucleotide sequences and specific parameters were created for each studied gene and miRNA. The binding site prediction programs only work with the textual format of the data presented in the files with the mir and gene extensions.
	8.4 Important statements are confirmed / partially confirmed / not confirmed by references to current and reliable scientific literature	The main important statements of the dissertation work are confirmed in this dissertation work by appropriate references to the extensive scientific literature.
	8.5 Used literature sources are sufficient /not	The author of the thesis has done a great deal of work in writing a

		sufficient for a literature review	review of the scientific literature on the research topic. The author used 422 publications devoted to the research topic. These sources of information are quite sufficient to substantiate the purpose and objectives of the study and show the relevance of the chosen topic.
9	Practical value principle	9.1 The thesis has theoretical value: 1) yes ; 2) no	The dissertation is of theoretical significance since the applicant obtained the following results previously unknown: the TCP, HSF, ERF, GRAS, MYB, C2H2 family target genes of the <i>T. aestivum</i> , <i>O. sativa</i> , <i>Z. mays</i> , and <i>A. thaliana</i> for miRNAs have been predicted; for some miRNAs, binding sites in mRNAs of the target genes of different plants are conservative and encode conservative oligopeptides; some miRNAs may have more than one binding sites in one mRNA and for than one target gene; mRNAs of some target genes may have binding sites for two or more different miRNAs; miRNA binding sites of plants may be located in the 5'UTR, CDS, and 3'UTR regions; miRNAs, which may bind to mRNAs of genes from different plant transcription factor families, have been identified
		9.2 The thesis is of practical importance and there is a high probability of applying the results obtained in practice: 1) yes ; 2) no	The dissertation is of practical importance and there are possibilities of applying the obtained results in activities of economic value. Most of the studied target genes of zma-miRNA, tae-miRNA, osa-miRNA may influence on human genes which participate in the development of oncological, neurodegenerative, and cardiovascular diseases.
		9.3 Are the practice suggestions new? 1) completely new ; 2) partially new (25-75% are new); 3) not new (less than 25% are new)	The given practice suggestion are new. The identified plant miRNAs can affect human genes and can be used as biologically active compounds, since they easily penetrate into the human body, which effectiveness has been revealed in several publications by foreign authors. These proposals are also confirmed by scientific articles published by the applicant in foreign peer-reviewed rating scientific journals.
10.	The quality of writing and design	Academic writing quality: 1) high ; 2) average; 3) below average; 4) low.	The dissertation work was performed at a high professional level, meeting the requirements. The thesis consists of three sections containing the results of research in accordance with the tasks set in the work. The literature review covers the information on the role and significance of the goal in studying the effect of miRNAs on

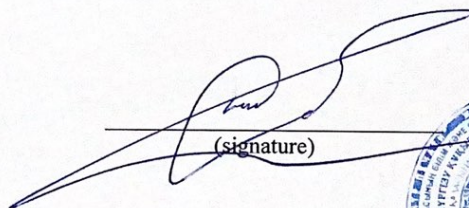
			<p>transcription factors of the most important agricultural crops. The formulation of the tasks to be achieved is fully justified. The presentation of the research results was carried out competently and convincingly shows the knowledge efficiency of the dissertation candidate.</p>
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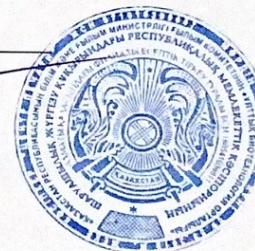
I consider that the dissertation of Rakhmetullina A.K. on the topic «Characteristics of miRNAs binding with mRNAs of transcription factor genes of agricultural plants» meets the requirements for a Ph.D. thesis in the specialty 6D070100-Biotechnology, and the candidate deserves a PhD degree.

Official Reviewer:

Candidate of Science (PhD),
Deputy Director,
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(place of work, academic title)


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Отв. по вопросам Манушев Ф.Р.* 