

**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>The completed dissertation research corresponds to the priority areas of the Science of Life and Health:</p> <p><b>5.1 Biotechnology in agriculture and environmental protection.</b></p> <p>5.1.1 Genomic editing technology, marker-related and genomic selection to improve economically valuable traits of plants and animals. – Almost each of the objectives of the dissertation includes the identification of associations of miRNAs and genes, which are markers of economically valuable traits of agricultural plants.</p> <p>5.1.8 Information technology in biology, agriculture and ecology. – In the thesis, information technologies are used to establish the regulation of key physiological processes of agricultural plants based on the use of miRNA.</p> <p><b>5.2 Biotechnology in medicine:</b></p> <p>5.2.2 Molecular, genomic, cellular and bioinformation technologies for the development of applied biology of personalized medicine. – The dissertation used molecular and bioinformatics technologies for the development of applied biology (biotechnology).</p> <p><b>5.3 Development of domestic pharmaceutical science and industrial biotechnology.</b> – The plant miRNAs associated with productivity identified in the dissertation can be used in industrial biotechnology and for the development of medicines.</p> <p>5.3.2 Technologies for obtaining valuable components from plant, animal and mineral raw materials using biotechnological methods. – The dissertation shows that miRNAs contained in plants can affect the synthesis of human proteins, and are valuable components of plant raw materials. The identified plant miRNAs can affect the</p>



			expression of genes that cause human diseases and can be used as therapeutic agents.
		<p>1) <b>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)</b></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>	The dissertation work was carried out within the framework of the 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 (2018-2020).
2.	Importance for science	The work <b>makes</b> /does not make a significant contribution to science, and its importance is <b>well disclosed</b> /not disclosed	The work makes a significant contribution to the development of agricultural science and the importance of this dissertation research is well disclosed in the work. The author has carried out studies, the results of which allow solving a specific and practically significant scientific problem - the creation of highly productive stress-resistant varieties of wheat, rice and corn using miRNA.
3.	The principle of independence	Self-reliance level: 1) <b>High</b> ; 2) Medium; 3) Low; 4) No independence	As part of the work on this topic, Rakhmetullina A.K. showed a high level of independence, she obtained a number of important and new results. The dissertation candidate independently substantiated and proved the scientific provisions submitted for defense, justifying the relevance of the research topic.
4.	The principle of inner unity	4.1 Justification of the relevance of the thesis: 1) <b>Justified</b> ; 2) Partially justified; 3) Not justified.	The relevance of the thesis is fully justified. Data on changes in the concentration of miRNA in cells at different stages of ontogenesis and under various influences are rapidly increasing, which confirms their important role in plant functioning. The use of miRNAs as modifiers of gene expression regulation is promising; however, it is necessary to accurately identify the target genes. The establishment of miRNA associations and their target genes will enhance the efficiency of plant genetic engineering. In this regard, the topic of the

			dissertation work by Rakhmetullina A.K., devoted to the study of the interaction of miRNA with mRNA of genes of agricultural plants, is relevant.
		4.2 The content of the thesis reflects the topic of the thesis: 1) <b>Reflects</b> ; 2) Partially reflects; 3) Does not reflect	The content of the dissertation fully reflects the topic of the dissertation. The dissertation is presented with an introduction, a review of the literature, materials and methods, results and discussion of experimental data, a conclusion, a list of used sources and appendices. Relevant reasoned conclusions are provided after each section.
		4.3. The purpose and objectives correspond to the topic of the thesis: 1) <b>correspond</b> ; 2) partially correspond; 3) do not correspond	The goals and objectives correspond to the topic of the thesis, suggesting to study the quantitative characteristics of the interaction of miRNA with mRNA of transcription factor genes of <i>A. thaliana</i> , <i>O. sativa</i> , <i>Z. mays</i> and <i>T. aestivum</i> and to identify the most effective associations of miRNA and target genes. To determine 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) <b>completely interconnected</b> ; 2) the interconnection is partial; 3) there is no interconnection	The dissertation is holistic research, all sections of which are completely interconnected and characterized by a logical sequence. I would like to note that since the studied plants are the most widespread agricultural crops and are used in human nutrition, author studied the effect of miRNAs of these plants on the regulation of human genes expression, including transcription factors.
		4.5 The new solutions (principles, methods) proposed by the author are reasoned and evaluated in comparison with the known solutions: 1) <b>there is a critical analysis</b> ; 2) partial analysis; 3) the analysis does not represent one's own opinions, but quotes from other authors	To solve the problems of creating agricultural plants with improved properties, as well as resistant to abiotic and biotic factors, the author, on the basis of a critical analysis of existing methods, proposes new methods for increasing plant productivity based on the use of miRNA. The proposed new solutions (principles, methods) are reasoned enough and objectively evaluated in comparison with the known solutions.
5.	Scientific novelty principle	5.1 Are the scientific results and provisions new? 1) <b>completely new</b> ;	The main completely new scientific results and five provisions of the dissertation work include: 1. The groups of target genes for miRNAs among TF families TCP,

		<p>2) partially new (25-75% are new); 3) not new (less than 25% are new)</p>	<p>HSF, MYB, GRAS, ERF, C2H2 of the <i>A. thaliana</i>, <i>O. sativa</i>, <i>Z. mays</i> and <i>T. aestivum</i> have been predicted for the first time; 2. For the first time binding sites of some miRNAs were identified in mRNAs of the target genes of different plants, that are conservative and encode conservative oligopeptides; 3. For the first time, it was revealed that some miRNAs can have more than one target gene and mRNAs of some target genes contain binding sites for two or more different miRNAs; 4. The binding sites of some miRNAs in CDS mRNA of plant TF genes can encode different oligopeptides depending on the reading frame; 5. For the first time, the binding sites of miRNAs of rice, maize and wheat with mRNA genes involved in various human diseases have been established. The novelty of the provisions proposed by the candidate for the defense is justified by publications in rating journals.</p>
		<p>5.2 Are the dissertation findings new? 1) <b>completely new</b>; 2) partially new (25-75% are new); 3) not new (less than 25% are new)</p>	<p>The conclusions of the dissertation are completely new, based on the research results, they are specific and justified:</p> <p>1. 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>, respectively. – This conclusion demonstrates the amount of work done to determine the number of miRNA and TF target genes in the studied plants.</p> <p>2. Based on the quantitative characteristics of the interaction of 428 ath-miRNAs and mRNA of 27 TCP family genes of <i>A. thaliana</i> revealed only 11 target genes for five miRNAs. The ath-miR5021-5p binding sites were found in mRNAs of three genes of <i>A. thaliana</i> and in mRNAs of 27 genes of 17 plant species. The ath-miR5658-5p binding sites in mRNA of 23 genes of 19 plant species are conservative. Of 738 osa-miRNAs and mRNA of 22 TCP family genes of <i>O. sativa</i> only 14 genes were shown to be the targets for 17 miRNAs. The osa-miR2102-5p binding sites encode the AAAAAA</p>

			<p>and GGGGGG oligopeptides in mRNAs of ten genes of seven plant species and in mRNAs of eight genes of six plant species, respectively. The analysis of binding of 325 zma-miRNAs and mRNA of 46 TCP family genes of <i>Z. mays</i> predicted only seven target genes for 11 miRNAs. Of 125 miRNA and 28 mRNA of the TCP family genes of <i>T. aestivum</i> revealed only five tae-miRNAs have five mRNA target genes. The tae-miR319-3p binding sites encode the conservative oligopeptide QRGPLQS in the TCP TF in 54 plant species. The tae-miR444a-3p binding sites encode the STSETS oligopeptide in mRNAs of 29 genes of 28 plant species. – Here the results of the study of 53 genes of the TCP family in four plant species, which are targets of 38 miRNAs, are summarized. The identification of conserved oligopeptides encoded by miRNA binding sites is covered in detail for the first time.</p> <p>3. Of 428 ath-miRNAs and mRNA of 24 HSF genes of <i>A. thaliana</i>, only five genes were targets for five miRNAs. Of 738 osa-miRNAs and mRNA of 25 HSF family genes of <i>O. sativa</i> 12 genes were under the control of ten miRNAs, of which the largest number of binding sites had miR5075-3p. Of 325 zma-miRNAs and mRNA 28 HSF family genes of <i>Z. mays</i> it was revealed only six target genes for seven miRNAs. Of 125 tae-miRNAs and the mRNA of 51 genes of the <i>T. aestivum</i> HSF family, it was found that only three genes of the HSF family were targets for four miRNAs. – This conclusion reflects the new results of interaction miRNA with mRNA of the HSF transcription factor genes of arabidopsis, rice, corn, and wheat.</p> <p>4. Out of 428 ath-miRNA binding sites in the mRNAs of 144 MYB genes of <i>A. thaliana</i> it was revealed that 32 genes are targets of 15 miRNAs. Of 738 osa-miRNA and mRNA of 124 genes of the MYB <i>O. sativa</i> family revealed that 34 genes were targets for 32 miRNAs. Of 325 zma-miRNAs and mRNA of 169 genes of the <i>Z. mays</i> MYB family, 25 genes were the targets for 26 miRNAs. Of 125 tae-miRNAs binding and mRNA of 258 MYB family genes of <i>T. aestivum</i> revealed that only eight genes were targets for eight</p>
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			<p>miRNAs. The tae-miR159a,b-3p binding sites encode the WSSIRSK oligopeptide, which is conserved in the 27 proteins of the MYB TF for 22 plant species. The proteins of the MYB family of 22 plant species contained the ELPSNQ oligopeptide encoded by miR159e-3p binding sites in mRNAs of 23 genes of 20 plant species. – This conclusion reflects the new results of interaction miRNA with mRNA of the MYB transcription factor genes of arabidopsis, rice, corn, and wheat, including information on the conservatism of miRNA binding sites and oligopeptides encoded by them in 22 plant species.</p> <p>5. Of 428 ath-miRNAs and mRNA of 37 genes of the <i>A. thaliana</i> GRAS family, it was found that only 11 genes were targets for eight miRNAs. Of 738 osa-miRNAs, only 16 miRNAs could bind to mRNA of 18 genes from 60 genes of the GRAS <i>O. sativa</i> family. Of 325 zma-miRNAs and mRNA of 86 genes of the GRAS family of <i>Z. mays</i>, only 14 genes were targets for eight miRNAs. Of 125 tae-miRNAs interaction with mRNA of 117 GRAS TF genes of <i>T. aestivum</i> revealed only five target genes for three miRNAs. The nucleotide sequences ath-miR171a-3p, osa-miR171a-3p, zma-miR171n-3p were identical and had binding sites in mRNA for 13 genes of the TF GRAS family and encode conservative ILARN oligopeptide. – In the conclusion, especially interesting data were revealed on the fully complementary interaction of miR171a-3p with mRNA of the GRAS transcription factor genes of arabidopsis, rice, corn, and wheat. The nucleotide sequences of ath-miR171a-3p, osa-miR171a-3p, zma-miR171n-3p were identical and had binding sites in mRNA of <i>A. thaliana</i> - 3 genes, <i>O. sativa</i> – 4 genes, <i>Z. mays</i> – 6 genes of GRAS family. The presented data indicate a high conservation of miR171 nucleotide sequences in three phylogenetically distant plant species, but retaining the interaction between miRNA and target genes. The analysis of the functions of the studied target genes carried out in this work showed that miR171 can regulate the expression of genes involved in seed formation.</p>
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			miRNAs interaction with mRNA of 17508 human genes revealed only 116 target genes for 44 single tae-miRNAs and 57 target genes for 23 tae-miRNA families. Among 17,508 human genes with 738 osa-miRNAs, 942 target genes for 277 osa-miRNAs were established. A total of 641 target genes were identified for 131 single miRNAs. 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. - The author was able to identify miRNAs of maize, rice, wheat, which can potentially significantly affect the expression of human genes involved in various socially significant diseases.
		5.3 Technical, technological, economic or management decisions are new and reasonable: 1) <b>completely new</b> ; 2) partially new (25-75% are new); 3) not new (less than 25% are new)	Technological solutions to the defined goals and objectives are completely new and justified. The bioinformatics technologies used made it possible to obtain many original results with novelty. The use of these technologies is justified, since they are more effective in solving the problems of the dissertation work.
6.	The validity of the main findings	All main conclusions <b>are based</b> /are not based on scientifically significant evidence or well-grounded (for qualitative research and areas of training in the arts and humanities)	All conclusions are based on scientifically sound evidence and reasonably well founded. This was achieved by the application of bioinformatics technologies and adequate approaches to studying the set goals and objectives.
7.	The main provisions for the defense	It is necessary to answer the following questions for each provision separately: 7.1 Is the provision proven? 1) <b>proven</b> ; 2) rather proven; 3) rather not proven; 4) not proven	The provisions for the defense have been proven. The applicant makes a number of fundamental statements for defense, which together represent a solution to the problem: creating the basis for obtaining highly productive stress-resistant varieties of wheat, rice and corn using miRNA. 1. 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 data on the quantitative characteristics of interaction miRNAs with genes of the of TCP, HSF, MYB, GRAS, ERF, C2H2 families TF of <i>A. thaliana</i> , <i>O. sativa</i> , <i>Z. mays</i> and <i>T. aestivum</i> .



		<p>2. The results of 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. – The proposed provision is based on the results of studying the effect of miRNAs on genes involved in plant growth and development. miRNAs that affect key genes of the families of transcription factors (TCP, HSF, MYB, GRAS, ERF, C2H2) have been identified.</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. – The author demonstrated that plant miRNAs can bind to mRNA genes at both the 5'UTR and 3'UTR, contradicting the idea that they can only bind at the CDS.</p> <p>4. 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. – Due to the quantitative characteristics of the interaction of miRNA with mRNA, the author was able to evaluate in a comparative aspect the interaction of various miRNAs with mRNA of target genes.</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. – The author presents the results of the interaction of plant miRNAs with mRNAs of human genes, which show the possibility of a significant effect of exogenous plant miRNAs on the human body.</p>	
		<p>7.2 Is it trivial?  1) yes;  2) <b>no</b></p>	<p>The provisions for the defense are not trivial. There are no elements of triviality in this dissertation work. All discovered patterns and features of the researched processes were considered not oversimplified, but from the perspective of modern knowledge in the field of biotechnology.</p>



		7.3 Is it new? 1) <b>yes</b> ; 2) no	The provisions for the defense are new. Previously, no one has described similar provisions and research results on the dissertation's topic in the literature.
		7.4 Application level: 1) narrow; 2) medium; 3) <b>wide</b>	The application level of the results of the dissertation work is wide, since most of the studied miRNAs and their target genes of transcription factors are homologous in many species of agricultural plants.
		7.5 Is it proven in the article? 1) <b>yes</b> ; 2) no	The proof of all provisions is confirmed by the publication of research results in 17 printed works, 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.
8.	The principle of reliability Reliability of sources and information provided	8.1 Choice of methodology - is justified or the methodology is described in sufficient detail 1) <b>yes</b> ; 2) no	The choice of research methodology is carefully justified; the research methods used are described in detail in the corresponding chapter of the thesis. All the results of the thesis were obtained using modern computer technology.
		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) <b>yes</b> ; 2) no	The miRNA binding sites in mRNAs of the target genes were predicted with the MirTarget program. The MirTarSeq program (The copyright certificate "MirTarSeq" No. 15600 dated March 2, 2021) was used to search for oligopeptides encoded by the nucleotide sequences of miRNA binding in CDS of mRNA genes.
		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) <b>yes</b> ; 2) no	The conclusions obtained in the dissertation research are proved and based on original, computational experimental data. The features of the interaction of miRNA with mRNA of genes of various families of transcription factors of arabidopsis, corn, rice, and wheat have been revealed.

		8.4 Important statements are <b>confirmed</b> / partially confirmed / not confirmed by references to current and reliable scientific literature	Important statements based on the data obtained are supported by references to reliable scientific literature. References to sources used are correct, most of them are publications of recent years.
		8.5 Used literature sources are <b>sufficient</b> /not sufficient for a literature review	The dissertation work contains a sufficient number of references to sources, consisting of 422 citations, including the works of foreign and local scientists.
9	Practical value principle	9.1 The thesis has theoretical value: 1) <b>yes</b> ; 2) no	The dissertation is of theoretical importance, since groups of target genes for miRNAs among TF families TCP, HSF, MYB, GRAS, ERF, C2H2 of the <i>A. thaliana</i> , <i>O. sativa</i> , <i>Z. mays</i> and <i>T. aestivum</i> have been predicted for the first time. For the first time, the binding sites of miRNAs of rice, maize and wheat with mRNA genes involved in various human diseases have been determined.
		9.2 The thesis is of practical importance and there is a high probability of applying the results obtained in practice: 1) <b>yes</b> ; 2) no	The establishment of plant miRNA associations and their target genes TFs of the TCP, MYB, GRAS, ERF, C2H2 families reduces the time and material costs of searching for connections between miRNAs and target genes of agricultural plants, compared to finding associations of miRNAs with mRNAs experimentally. The revealed associations of miRNA and TF target genes in the studied plants allow targeted modification to improve many useful properties of various species of plants. MirTarget program has been successfully used to find miRNA binding sites in mRNAs of plant and human genes, and to quantify the interaction of miRNA with mRNA target genes. The identified plant miRNAs can influence human genes and be used in medicine as biologically active compounds, since they easily penetrate the human body. The copyright certificate "MirTarSeq" No. 15600 dated March 2, 2021 was received.
		9.3 Are the practice suggestions new? 1) <b>completely new</b> ; 2) partially new (25-75% are new); 3) not new (less than 25% are new)	Practical suggestions and recommendations have a high level of originality. The established associations of miRNAs and target genes can be used to create plant varieties that are highly productive and resistant to abiotic and biotic stresses. The results obtained in this work help to elucidate the possible role of plant miRNAs in the regulation of human gene expression.



10.	The quality of writing and design	Academic writing quality: 1) <b>high</b> ; 2) average; 3) below average; 4) low.	The conducted research presents the ability of a doctoral student to analyze information and draw appropriate conclusions. The work is completely framed in accordance with the academic research format and requirements. The dissertation writing is of high quality.
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By considering the thesis presented by A.K. Rakhmetullina on the topic "Characteristics of miRNAs binding with mRNAs of transcription factor genes of agricultural plants", presented for the degree of Doctor of Philosophy (PhD), I agree to award the degree of Doctor of Philosophy (PhD) in the specialty 6D070100-Biotechnology.

**Official Reviewer:**

Laboratory of molecular biology  
Institute of plant biology and biotechnology  
MES RK  
 (place of work, academic title)



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 (signature)

Dilvara Gritsenko  
 (FULL NAME)

