Original Article

In silico analysis of maize and wheat miRNAs as potential regulators of human gene expression

Aizhan Rakhmetullina[1,2]^, Anatoliy Ivashchenko[3]^, Anna Pyrkova[3]^, Kairat Uteulin[4]^, Piotr Zielenkiewicz[1]^

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]Department of Technology of Production of Livestock Products, Baitursynov Kostanay Regional University, Kostanay, Kazakhstan; [3]Department of Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan; [4]Laboratory of Cell Engineering, Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan

Contributions: (I) Concept and design: P Zielenkiewicz, A Ivashchenko, A Rakhmetullina; (II) Administrative support: A Pyrkova, K Uteulin, P Zielenkiewicz; (III) Provision of study materials or patients: A Ivashchenko, A Rakhmetullina; (IV) Collection and assembly of data: A Pyrkova, K Uteulin; (V) Data analysis and interpretation: A Ivashchenko, A Rakhmetullina; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Prof. Piotr Zielenkiewicz, PhD, DSc. Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 02-106 Warsaw, Poland. Email: piotr@ibb.waw.pl.

Background: Human consumption of food and herbal medicines supplies the body with not only nutrients but also associated biologically active compounds, such as exogenic messenger RNA (mRNA) inhibitory RNAs (miRNAs). Therefore, it seems important to establish which maize and wheat miRNAs can enter the human body and evaluate their possible actions and binding features with human mRNA genes. It is required to determine whether the most common plant miRNAs can interact with human genes and what consequences this interaction may have.

Methods: The quantitative parameters of interactions of plant miRNAs with candidate human mRNA genes were determined using the MirTarget software, which defines the following features of plant miRNA binding to mRNA: the location of plant miRNA binding sites (BSs) in mRNA; the localization of the plant miRNA BSs in the 5'-untranslated region (5'UTR), coding domain sequence (CDS), and 3'-untranslated region (3'UTR) of the mRNAs; the free energy of interactions between plant miRNA and mRNA, and nucleotide interaction schemes between plant miRNAs and mRNAs.

Results: The miRNAs from maize and wheat might have effective BSs in the mRNA of human genes. Some miRNAs have potential to bind to the mRNAs of one or more human genes. Important features of the interaction of plant miRNAs and mRNA nucleotides at the 5- and 3-ends of BSs were revealed, indicating that the interactions of these molecules are conserved. As a result, in order to use identified wheat and maize miRNAs in medicine, experimental validation in combination with our *in silico* studies will be beneficial to understand the impact of these miRNAs on changes in human gene expression levels.

Conclusions: Plant miRNAs include miRNAs that are most actively involved in plant growth and development. The target genes of most plant miRNAs interact with human genes involved cancer development, neurodegenerative diseases, cardiovascular diseases, diabetes, and autism. The miRNAs of maize and wheat can be used as regulators of expression of many processes in the human body.

Keywords: Plant messenger RNA inhibitory RNAs (plant miRNAs); binding sites (BSs); maize; wheat; human genome

Received: 30 March 2023; Accepted: 25 August 2023; Published online: 27 September 2023.
doi: 10.21037/exrna-23-4
View this article at: https://dx.doi.org/10.21037/exrna-23-4

^ ORCID: Piotr Zielenkiewicz, 0000-0001-8151-6874; Aizhan Rakhmetullina, 0000-0002-0117-7110; Anatoliy Ivashchenko, 0000-0002-7969-2016; Anna Pyrkova, 0000-0001-8483-451X; Kairat Uteulin, 0000-0002-5459-0902.

Introduction

Plant messenger RNA (mRNA) inhibitory RNAs (miRNAs) are nanoscale noncoding RNAs 19–24 nucleotides long that regulate the expression of genes in animals (1) and plants (2,3) at the posttranscriptional level. These molecules can regulate the expression of target genes by cleaving mRNA, or they can prevent translation (4,5). Many plant miRNAs are crucial in the control of plant growth, development, and tolerance to biotic and abiotic stresses. In both plants and animals, miRNAs are key posttranscriptional regulators of gene expression.

Since a number of publications describe the entry of plant miRNAs into the human body through food intake (6), it is important to determine the possibilities of interaction between plant miRNAs and human genes. It was shown that that exogenous plant miRNA ingested by mammals can survive through the gastrointestinal system, and enter the bloodstream and various organs of mice (7). Liang *et al.* detected a variety of fruit miRNAs in the human plasma after feeding recruited volunteers with watermelon juice or mixed fruits (8). Liu *et al.* provided a novel information for the research and application of soybean-derived miRNAs in cancer occurrence (9). A recent study showed that olive small RNA is functionally homologous to human miRNA34 in cross-kingdom regulation of tumorigenesis (10). It has been demonstrated that plant small RNAs from Moringa oleifera may restore normalcy in immune system and reduce replication of human immunodeficiency virus (HIV) infection (11). Such small non-coding RNAs have also recently been used as tumor suppressor RNAs to play a role in tumor progression (12). The available information on the possible effect of plant miRNAs on human physiological processes is not sufficient for the target-based utilization of certain miRNAs for specific genes (13,14). Plant miR159 was previously shown to inhibit cancer growth in mammals (15). According to Zhang *et al.*, rice-derived miRNA-168a regulates gene expression effectively and has been shown to target LDLRAP1 protein, a gene involved in cholesterol metabolism (16). It has been demonstrated that plant miRNAs have a regulatory effect on human genes involved in lipid metabolism (17). Plant mir156a has been shown to have an atheroprotective effect on inflamed human endothelial cells (18). Plant mir167e-5p can inhibit enterocyte proliferation by acting on β-catenin (19-21). The role of plant miRNAs as an immunomodulator in humans and animals has been revealed (22). Cavalieri *et al.* from the same study suggest that plant miRNAs can prevent chronic-inflammation related diseases (22).

The cross-kingdom regulation of miRNAs has become an attractive topic in nutrition science due to their ingestion with food (23-27). Plant miRNAs have the capacity to be stored in the gastrointestinal tract (28), enter the bloodstream, and control the production of protein synthesis via interactions with mRNA (1,23,24). Exogenous miRNAs can diffuse through body fluid, allowing cross-kingdom delivery (7,16,29,30). After surviving digestion, miRNAs use extracellular vesicles (31), e.g., exosomes (32-34), vesicles (35), specific lipoproteins (36), some viruses and Argonaute-containing bodies (37), to enter the bloodstream. “Trancytosis” is another process by which miRNAs can enter body fluids and involves the formation of a vesicle from the intestinal wall (38). Immune system cells (39) and specific transporters (40,41) may also help in this transportation. Previous studies have shown the slight reduction in miRNA content that occurs during storage, processing, and preparation of plant-based foods is one factor impacting the entry of plant miRNA into the human body (16,42). The potential of exogenous plant miRNAs to control target genes both in animals and humans opens up new possibilities for the treatment of human diseases using ex-miRNAs during therapy (43-45). Yang *et al.* have shown that dietary sRNAs could survive circulation and are excreted in urine (30). miR172, the most abundant miRNA in cabbage, was found in mice stomachs, serum,

Highlight box

Key findings

- Corn and wheat microRNAs (miRNAs) have a potential to interact with messenger RNA (mRNA) of human genes.

What is known and what is new?

- Human consumption of food and herbal medicines supplies the body with not only nutrients but also associated biologically active compounds, such as exogenic miRNAs. Numerous miRNAs from maize and wheat might have effective binding sites (BSs) in the mRNA of human genes. Many zma-miRNAs and tae-miRNAs can bind to mRNAs of target genes of various human diseases. Given the high conservation of some miRNAs in many plant species, one should expect edible plants to influence the expression of human genes.

What is the implication, and what should change now?

- As a result, after experimental verification of our *in silico* results the diets of both healthy and sick people should be changed to accommodate the knowledge on impact of edible plants miRNAs on the expression of human genes.

intestines, and feces (23). This study showed that ingested plant miRNAs may be involved in metabolism comparable to endogenous miRNAs, and with continuous feeding, the miRNA concentration can be consistent for few days (8,42). Following the consumption of watermelon juice, ten exogenous plant miRNAs were discovered in human plasma (8,46). After feeding maize for seven days, plant miRNAs were detected in pig tissues and blood (42). Human sera has been found to contain the plant-derived miR159, which was shown to target the *TCF7* gene and prevent the development of breast cancer (15). The research revealed that orally administered plant miRNAs have the potential to be found in human sera and could affect the development of cancer in mammals *in vitro*. It was discovered that the exogenous plant miR168a was present in animal and human plasma and that miR168a regulated the expression of the human *LDLRAP1* gene (16). miR156, one of the most conserved miRNAs in plants, is highly expressed in various crops, including wheat (47) and maize (48). In 2018, Hou *et al.*, revealed that exogenous miR156a from green vegetables could be detected in human serum and showed the involvement of plant miR156a in cardiovascular diseases (18). Broccoli-derived miR156a has been shown to have therapeutic potential for the therapy of nasopharyngeal cancer patients (49). This result is confirmed by MirTarget program in the present work. Other findings indicate that miR156 inhibits intestinal cell proliferation by targeting *Wnt10b* (20).

The diversity of miRNAs in each plant species is great, however, in each of them there are identical key miRNAs that determine the regulation of plant growth and development (50,51). Previous evolutionary analyses have reported the functional and evolutionary significance of miR156 in land plants (52). Yang *et al.* proposed a work model for Arabidopsis leaf development and drought tolerance mediated by miR160 and miR165/166 interactions (53). Recently, bioinformatics approaches have been successfully used to predict the effect of ex-miRNAs on the mRNA of human genes, which reduces the amount of work and materials needed to determine how miRNAs interact with mRNAs (54-56). Computational approaches have been assessed for *Hypericum perforatum* flower miRNAs in relation to cancer. It was demonstrated that some of these miRNAs potentially have a significant and critical tumor-suppressive role for prostate cancer (57). *In silico* results indicated the role of miR156, miR414 and miR5021 in essential oil biosynthesis by the modulation of terpenoid backbone synthesis pathways (58). Although plant miRNAs have the potential to regulate gene expression, it is important to correctly find the target genes and prevent negative impacts (59). A number of studies have shown that miRNAs of different plants can enter the human body and circulate in the blood together with endogenous miRNAs, but the target genes of these miRNAs were not specified. The above data on the detection of plant miRNAs in the human body and their possible effect on gene expression indicate the need for studies on the effect of plant miRNAs on human genes. The aim of our research was to examine the interaction of human genes with *Zea mays* miRNAs (zma-miRNAs) and *Triticum aestivum* miRNAs (tae-miRNAs), which are some of the most valuable and high-yielding cereals cultivated on all continents and are the staple food for approximately one-third of the world's population. These plant miRNAs might be used in future medicine as therapeutic agents in the regulation of the expression of target genes involved in a variety of biological processes in human body (60-62).

Methods

GenBank database (http://www.ncbi.nlm.nih.gov) was used to collect 17,508 human mRNA nucleotide sequences. Mature plant miRNA sequences were obtained from miRBase repository (v.22) (http://www.mirbase.org). The WebLogo (63,64) program was used to assess nucleotide sequence variability (https://weblogo.berkeley.edu/logo.cgi). The MirTarget software was used to search for the target genes of zma-miRNAs and tae-miRNAs (65,66). The MirTarget program determines the following binding characteristics: the start of the plant miRNA binding site (BS) of mRNA; the localization of plant miRNA BSs [5'-untranslated region (5'UTR); coding domain sequence (CDS) and 3'-untranslated region (3'UTR)]; the nucleotide interaction schemes between plant miRNAs and mRNAs and interaction free energy (ΔG, kJ/mol). The ΔG value depends on GC-content and the number of nucleotides in miRNA. For each BS, the ΔG/ΔGm (%) ratio was found, where ΔGm is equal to the free energy binding of plant miRNA with its full complementary nucleotide sequence. The MirTarget software identifies hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, and A and C. The distances between A and C (1.04 nm) and G and U (1.02 nm) bonds are comparable to the 1.03 nanometer distance between A and U, G and C (59-61). The number of hydrogen bonds in the G-C, A-U, G-U and A-C interactions has been found to be 3, 2, 1 and

Table 1 Characteristics of single zma-miRNA interactions with mRNA of human target genes

zma-miRNA	Gene	ΔG, kJ/mol	ΔG/ΔGm, %	Length, nt
miR162-5p	*ZNF853*	–104	91	21
miR162-3p	*DNAH3, PLXNA3, RIMS3*	–98 to –96	92 to 94	20
miR482-5p	*CDKN1C, FAM168A, FGF17, KCNK16, ROBO4, SLC44A4*	–98 to –96	94 to 96	19
miR482-3p	*ATP2A3, CSF1, LARP1, LMBRD2, PTER, SLC35A2, STK32A, TMCO5A, TTC25*	–98 to –96	92 to 94	20
miR529-5p	*PPIE*	–102	92	21
miR529-3p	*ATP6V0A4, CHD2, LMO7, LRTOMT, LTB4R, MLL, NUDC, PDE4B, POMC, SOX9*	–106 to –102	91 to 94	21
miR827-3p	*AKAP11*	–100	94	21

mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; nt, nucleotide.

1, respectively (67-70). The adequacy of the MirTarget program in terms of finding BSs has been confirmed in several publications (71-73).

To support the selection of the MirTarget software, we analyzed the existing tools for searching for BSs between plant miRNA and mRNA. We used TAPIR (74), psRNATarget (75), and RNA 22 (76) to show the effectiveness of the MirTarget compared to other programs (Table S1). To determine whether predicted plant miRNA target genes are involved in the development of human diseases, the DisGeNET (http://www.disgenet.org) platform was used (77). DisGeNET combines information from catalogs, scientific journals, and archives that have been expertly curated. The functional analyses were generated through the use of Ingenuity Pathway Analysis (QIAGEN Inc., Venlo, The Netherlands; https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa/) (78).

Statistical methods

A total of 17,500 genes were searched—assuming 2,000 nt of sequence per transcript as an approximation that is about 35 million base pairs of sequence. The probability if having a particular 21-nt sequence is 421, i.e., 4.4×1,012. It can be noted, however, that our identified motifs either 9 or 10 perfectly conserved bases and a number of others that are partially conserved. As these number varies from sequence to sequence, the common expectation value is not the same for all these sequences. Instead, following a referee suggestion we assume the 14-mer as commonly found number of identities. The probability of finding certain n nucleotides in certain positions in the of k-mer sequence is equal to: probability of choosing certain n positions in k-mer sequence $\binom{n}{k}$ multiplied by the probability of finding correct nucleotides in these positions $\left(\frac{1}{4}\right)^k$.

$$P = \frac{1}{\binom{n}{k}} \times \left(\frac{1}{n}\right)^k \quad [1]$$

which for the 14-mer evaluates to $\frac{1}{116280} \times \left(\frac{1}{4}\right)^{14} = 3,20 \times 10^{-14}$.

Results

Characteristics of single zma-miRNA interactions with mRNAs of human target genes

We used NCBI GenBank data and novel bioinformatics tool to define miRNA binding parameters. Study of the interaction of 325 maize miRNAs with mRNAs of 17,508 protein coding genes indicated only 31 human target genes for nine maize miRNAs chosen based on the ΔG/ΔGm ratio (ΔG, the free energy of the interaction between miRNA and the mRNA; ΔGm equals the free energy of the miRNA binding with its fully complementary nucleotide sequence) equal to 90% and greater. The results attained are presented in *Table 1*. zma-miR162-5p, zma-miR529-5p, and zma-miR827-3p were found to have only one target gene. Many of the discovered target genes for maize-derived miR529-3p perform a variety of functions in cells and are essential for controlling numerous processes in the human organism (table available at https://cdn.amegroups.cn/static/public/exrna-23-4-1.xlsx). All identified interactions of zma-miRNAs with mRNA of human genes had a free binding

energy ranged from –96 to –106 kJ/mol (*Table 1*). The ΔG/ΔGm values were also more than 90%, which indicates that the interaction of miRNA nucleotides and human gene BSs occurs mainly due to canonical nucleotide pairs.

The ΔG/ΔGm ratio for maize miR482-5p BSs ranged between 94% and 96%, which shows that the interactions of zma-miR482-5p with mRNAs of human target genes have a high degree of complementarity (*Table 1*). The functions of the identified human target genes may have a role in the development of many diseases, including abdominal carcinoma, nonhematologic malignant neoplasm, thyroid carcinoma, etc. (table available at https://cdn.amegroups.cn/static/public/exrna-23-4-1.xlsx).

A wide range of interactions between maize-derived miRNAs and a number of human target genes may indicate a possible preventative effect of plant exogenous miRNAs on changes in human gene expression levels associated with various diseases. Particularly, the study of the therapeutic effect of medicinal plant miRNAs is being actively studied (60-62). The effect of both 5p and 3p strands of miRNAs on human target genes is one of the features of plant miRNAs. According to *Table 1*, both strands of maize-derived miR162, miR482, and miR529 can bind to the mRNA of several target genes. The human miRNA-5p and miRNA-3p from the same miRNA rarely have a similar number of target genes (71).

Interaction characteristics of zma-miRNA families with mRNAs of human target genes

Many plant miRNA sequences vary by only one or two nucleotides at the miR-5p or miR-3p, which is the basis for grouping plant miRNAs into families. Lettered suffixes are assigned to the name to indicate closely related mature miRNAs expressed from different precursors or genomic loci and signify miRNA family members (e.g., zma-miR160e-5p and zma-miR160g-5p) (79). With the most regular length of plant miRNAs (21–22 nt), the family members are 90% identical. The characteristics of the interaction of members of the zma-miRNA families with human mRNA genes are shown in *Table 2*. miRNAs from the same family had similar free binding energies with mRNA of human genes, since the length of their nucleotide sequences usually differed by only one nucleotide. Highly complementary interactions of zma-miRNAs with mRNA of target genes had miR169q-3p (ΔG/ΔGm was 94–98%), miR319a,c-5p (ΔG/ΔGm was 92–96%), miR399j-5p and miR528a,b-3p (ΔG/ΔGm was 91–96%).

The results obtained show that some of maize-derived miRNA families have from one up to 18 (miR408b-5p) human target genes, table available at https://cdn.amegroups.cn/static/public/exrna-23-4-1.xlsx lists the human target genes of maize miRNAs that might be involved in the development of deafness—*ADCY1*; lung cancer—*ARHGAP30*; glioblastoma multiforme—*CPNE6*; diabetes—*GCGR*; ischemic stroke—*GPR124*; mental depression—IL6R; liver injury—*MAFK*; gastric cancer—*MAP3K6*; morphological development for neuronal polarization and axon growth—*NELL2*; Alzheimer's disease—*PLXNA4*; hepatocellular carcinoma—*PPM1F*; cervical cancer—*PXN*; nasopharyngeal carcinoma—*RAB37*; aspirin-exacerbated respiratory disease—*WDR46*; and esophageal squamous cell carcinoma—*ZNF132*, among others. The presented list of target genes involved in numerous human diseases demonstrates the potential of maize miRNAs in the regulation of the pathogenesis of the indicated diseases. The majority of the human target genes have a role in the development of oncological, cardiovascular and neurogenerative diseases (table available at https://cdn.amegroups.cn/static/public/exrna-23-4-1.xlsx).

The interaction schemes of nucleotides in these molecules are the most important characteristic in determining how plant-derived miRNAs interact with the mRNA of their target genes. The interactions of zma-miR482-5p, zma-miR169n-3p and zma-miR166l,m-3p with the mRNA of *ROBO4*, *TRMT2A*, and *XAB2* human target genes are shown schematically in *Figure 1*. The figure shows that noncanonical A-C and G-U pairs enhance the free energy of interaction between plant miRNAs and human mRNAs. Furthermore, they maintain stacking interactions between nucleotides in each strand of the RNA helix (67,68).

Table S2 demonstrates the binding characteristics of maize miR529-3p to human mRNA genes, which show where miRNA BSs are located in the 5'UTR, CDS, and 3'UTR. It is essential for plant miRNA to have BSs in any of these target mRNA regions (Table S3).

WebLogo (63,64) schemes of the nucleotide sequence variability in the regions of human mRNA genes containing the BSs for maize miRNAs and wheat miRNAs show conserved BSs of several nucleotides at the 5- and 3-end. This demonstrates the significance of the 5- and 3-end in plant miRNAs binding to human mRNAs with higher GC content, which allows the binding of miRNAs with the highest free energy interaction (*Figure 2*).

Therefore, determining the BS based on only a few nucleotides from the 5-end of the "seed" is insufficient.

Table 2 Interaction characteristics of zma-miRNA families with mRNAs of human target genes

zma-miRNA	Gene	ΔG, kJ/mol	ΔG/ΔGm, %	Length, nt
miR156j-5p	*AP2A2*	–104	94	21
miR156i-3p	*SGSM1*	–110	95	22
miR159e-5p	*BZRAP1, FEN1, LZTS1, PDE6B, PRCD*	–106 to –104	91 to 93	21
miR159h,i-3p	*ATF6B, DEAF1, DGCR8, DOK6, GPSM3, TMEM229B, TTN, URM1*	–104 to –102	91 to 92	21
miR160a-e,g-5p	*DAB2*	–108	93	21
miR164f-5p	*ASXL1*	–110	95	21
miR160f-3p	*ALKBH5, BCL9L*	–110	91	21
miR164b-3p	*DYSF, EXOC7, HPD, ITGA7, PTPRF, ZNF37A*	–102 to –100	92 to 94	20
miR166b-i-3p	*CHRM1*	–102	92	20
miR166m-5p	*CCNY, HGS, INSM1, SGIP1, TNFSF13, TSNARE1*	–110 to –106	91 to 95	21
miR167e-j-5p	*HSF1, FAM43B, PRICKLE2*	–106 to –102	91 to 94	21
miR167j-3p	*FAM57A, FMNL2, GPR107*	–98 to –93	92 to 96	20
miR168a-3p	*FLAD1*	–102	92	20
miR169i,j,k-5p	*GRHL3, NCOA6*	–104	92	21
miR169q-3p	*ADRA1D, CA6, CYP1A1, DUOX1, EPHB6, MAP3K12, MARCH10, OXSR1, PIK3C2B, TRMT2A, TUBA3C, WNT16*	–104 to –100	94 to 98	19
miR171d,e-5p	*NFATC2, SIRT7*	–106 to –104	91 to 93	21
miR171g-3p	*BRD3*	–104	91	21
miR172a-d-3p	*GPR31, LEF1, TECTA, TUFM*	–96 to –93	92 to 94	20
miR172c-5p	*GON4L, LASP1, SLC30A8, YY1AP1*	–100 to –98	92 to 94	20
miR2118g-3p	*DYRK1A, XPO6*	–106 to –104	91 to 93	22
miR2275a-3p	*GPR22, SACS*	–102 to –100	92 to 94	22
miR2275d-5p	*AHCYL2, CER1, FAM205A, SPRR1B*	–100 to –98	92 to 94	21
miR319a,c-5p	*PLCD4, RNF157*	–104 to –100	92 to 96	20
miR319a-d-3p	*DMAP1, IL4I1*	–102	92	20
miR390a,b-5p	*ASPSCR1, HFE*	–108 to –106	91 to 93	21
miR390a,b-3p	*PDAP1*	–102	91	21
miR393a,c-5p	*TMEM136*	–106	93	22
miR393c-3p	*LRP1B, TP53I3*	–106	93	22
miR394a,b-5p	*BIN2, GRIK4, HAVCR2, HNF1B, IGSF3, MICAL3, PGAP1, REXO4, RXFP1*	–102 to –100	92 to 94	20
miR394a,b-3p	*ALDH4A1, CIB3, PURG*	–100	92	20
miR395a-j,n,p-3p	*CD22, EDN1, TEAD2*	–108 to –102	91 to 96	21
miR395k-5p	*CCDC117, MYOM1*	–106 to –104	92 to 94	22
miR396a,b-5p	*SCAMP5, SYVN1, VPS13B*	–102 to –100	92 to 94	21

Table 2 *(continued)*

Table 2 *(continued)*

zma-miRNA	Gene	ΔG, kJ/mol	ΔG/ΔGm, %	Length, nt
miR396g-3p	*EVC2*	–100	92	21
miR397a,b-5p	*PKP3*	–102	91	21
miR397b-3p	*C6orf223*	–106	93	21
miR398a,b-3p	*EPS8, NUAK1, PCDHGA12*	–108	91	21
miR398b-5p	*BAHCC1, NUP62*	–108	91	21
miR399d-3p	*APBB1, CDK18, DDX11, JAGN1, OSTM1*	–108 to –106	91 to 93	21
miR399j-5p	*AMMECR1L, CHTF18, EDN3, IKZF3, LARP4B, LDLRAD2, LGI4, SIAH3, SPSB3, SOX18, SPOCK2, TRAK1*	–115 to –108	91 to 96	21
miR408a,b-3p	*ADARB2, ADCY6, ALPK3, ANO4, EDEM1, ERICH1, FNIP2, KIRREL3, NGB, PAQR6, RBMS2, RLBP1, SMARCC2, TJP3, UBE2K, UNG*	–113 to –108	91 to 95	21
miR408b-5p	*ADCY1, ARHGAP30, CPNE6, DYRK1B, GCGR, GPR124, IL6R, MAFK, MAP3K6, NELL2, PLXNA4, PPM1F, PXN, RAB37, TMEM91, TNIP1, WDR46, ZNF132*	–110 to –108	91 to 93	21
miR444a,b-3p	*CEP250, CSRNP1, MPDZ*	–102 to –100	92 to 94	21
miR528a,b-5p	*CACNB1, CD276, DNAJB6, GSK3B, GTF3C1, PPP1R26, PROB1*	–108 to –106	91 to 93	21
miR528a,b-3p	*EXOC7, FREM2, LSM4, MC1R, MGAT3, NFE2L1, RANBP1, RNF14, SPAG5, UIMC1*	–110 to –104	91 to 96	21

mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; nt, nucleotide.

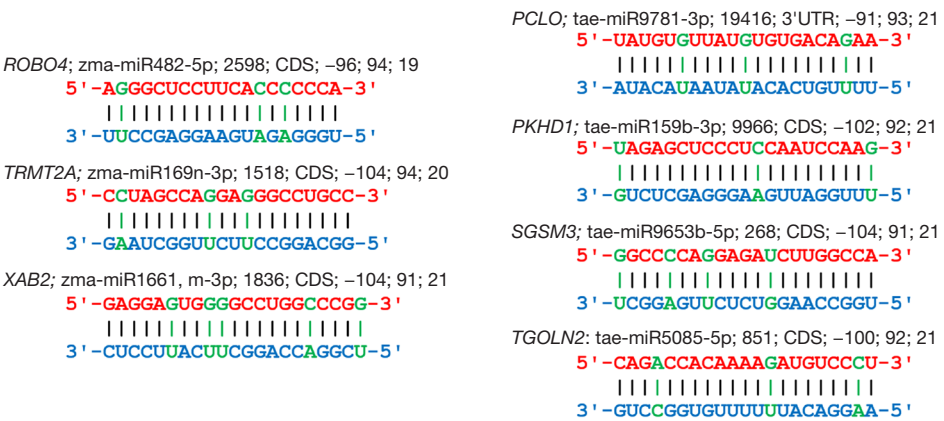

Figure 1 The schemes of nucleotide interactions of zma-miRNA and tae-miRNA with human mRNA genes. The header above each sequence contains: gene name; miRNA; start of binding site (nt); mRNA region; free energy ΔG (kJ/mol); ΔG/ΔGm (%); miRNA length (nt). Symbols defined in Methods section. The upper and lower nucleotide sequences of mRNAs (red) and miRNAs (blue), respectively. The green bold font represents the nucleotide of non-canonical U-G, A-C pairs. mRNA, messenger RNA; zma, *Zea mays*; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; nt, nucleotide; CDS, coding domain sequence.

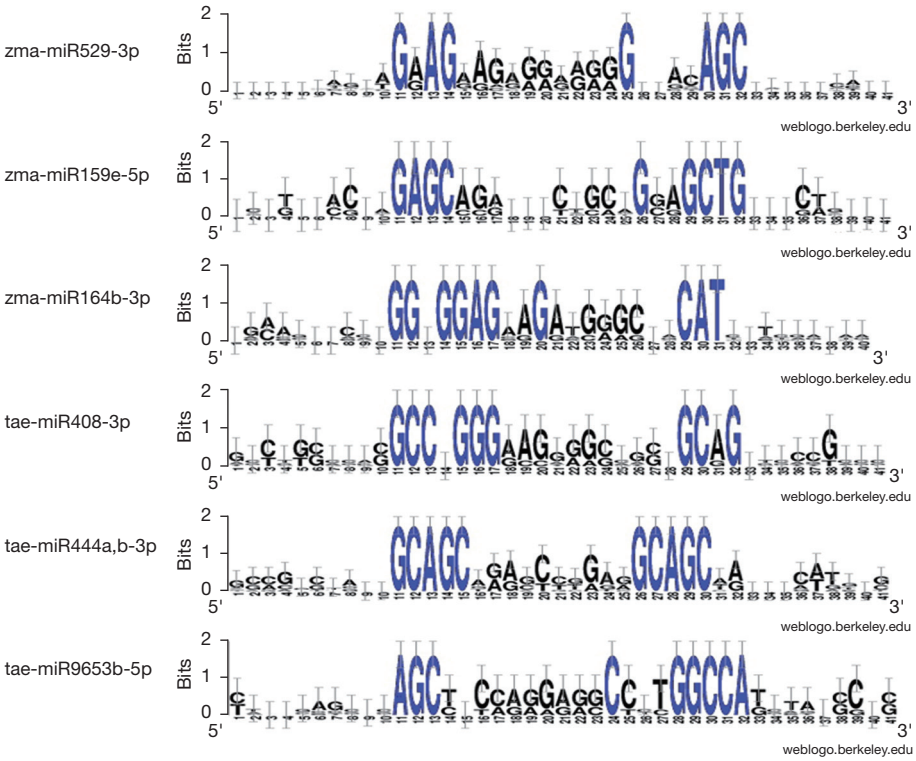

Figure 2 WebLogo schemes of the nucleotide sequence variability in the regions of human mRNA genes containing the BSs for zma-miRNA and tae-miRNA: zma-miR529-3p (10 genes); zma-miR159e-5p (5 genes); zma-miR164b-3p (6 genes); tae-miR408-3p (6 genes); tae-miR444a,b-3p (9 genes); tae-miR9653b-5p (6 genes). The nucleotides present in all miRNA BSs are highlighted in blue. BSs, binding sites; mRNA, messenger RNA; miRNA, mRNA-inhibitory RNA.

The study of the interaction of maize-derived miR159e-5p (Tables S4,S5) and miR164b-3p (Tables S6,S7) with human mRNA genes revealed similar results.

Characteristics of single tae-miRNA interactions with mRNAs of human target genes

Among the wheat miRNAs demonstrated in *Table 3*, miR156 (80,81), miR160 (82), miR164 (82), miR319 (83), miR396 (84), miR397 (85), miR398 (85), miR399 (83), miR408 (85,86), miR5048, miR9654b-miR9657b, miR9676-miR9679, miR9661, miR5384, miR9662a,b, and miR9652-5p (50) are found in many plants and are conserved. The free energy of interaction of miR169-3p, miR1119-3p, miR1129-5p, and miR9780-3p with mRNAs of the corresponding genes ranged from –113 to –129 kJ/mol, which indicates a strong interaction of these molecules (*Table 3*).

The discovered wheat miRNA target genes perform multiple functions in cells, and many of them are important in the regulation of crucial processes in the human body and the development of different diseases (table available at https://cdn.amegroups.cn/static/public/exrna-23-4-1.xlsx).

Interaction characteristics of tae-miRNA families with mRNAs of human target genes

The wheat miRNA families consist of only 2–3 tae-miRNAs that target one to nine human genes, as demonstrated in *Table 4*. The ΔG/ΔGm value varies from 91% to 98%, which indicates a high degree of complementarity between miRNA and mRNA nucleotides. The G/Gm ratio varied from 91% to 98%, which shows that the nucleotides of miRNA and mRNA are highly complementary to each other. The values of free energy of interaction between wheat miRNAs and human mRNAs were higher than –90 kJ/mol.

Table S8 shows the binding characteristics of wheat miR408-3p interaction with six human genes, indicating their effective binding. The sequences of regions of human mRNA genes containing wheat miR408-3p BSs reveal (Table S9) conserved nucleotides at the 5- and 3-end (*Figure 2*).

Table 3 Characteristics of single tae-miRNA interactions with mRNAs of human target genes

tae-miRNA	Gene	ΔG, kJ/mol	ΔG/ΔGm, %	Length, nt
miR156-5p	*AP2A2, ZNF652, F11R (JAMA)*	–104 to –102	90 to 94	21
miR160-5p	*C11orf16, DAB2*	–108 to –106	91 to 93	21
miR164-5p	*ASXL1, RASL10B, UGT1A7, UGT1A9*	–108 to –106	91 to 93	21
miR169-3p	*AARS2, GTPBP3, LYNX1*	–119 to –113	90 to 95	22
miR319-3p	*C15orf55, IL4I1, PTPMT1*	–104	91	21
miR396-5p	*AIM1*	–104	91	21
miR397-3p	*ING1*	–108	93	21
miR398-3p	*EPS8, NUAK1, PCDHGA12*	–108	91	21
miR399-3p	*HSPG2, JAGN1, U2AF2*	–98 to –96	94 to 96	19
miR408-3p	*ALPK3, RBMS2, EDEM1, NGB, PAQR6, UNG*	–113 to –108	91 to 95	21
miR531-5p	*SLC5A10*	–113	91	21
miR1118-5p	*DCLK1, HECTD3, MDP1, OR51E2*	–110	93	23
miR1119-3p	*HPDL, KCNA1, SLC27A3*	–121	90	24
miR1124-3p	*AGRP, ARVCF, CPN1, FNTA, SPRN, URB1*	–110	90	22
miR1129-5p	*CDAN1, ENTPD2, LMO3, SLC47A1, RAX, RERE, WDR45*	–129	92	24
miR1134-3p	*CCND2, NIPAL4, NOTCH2, TCF7L2, TMEM178B*	–108	91	24
miR1138-3p	*C21orf2, MFI2, MOG*	–104	91	23
miR1139-5p	*CDON*	–98	90	22
miR1847-5p	*ATRIP, HSD3B1*	–104	91	21
miR5048-5p	*FLCN*	–100	90	22
miR5050-5p	*MIER1*	–102	91	21
miR5084-3p	*RYR2*	–110	90	24
miR5085-5p	*TGOLN2, ZFHX3*	–100	92	21
miR5086-5p	*DHX30, HBP1, LTBP4, MYO1G, SERPING1, SPTAN1*	–104 to –102	91 to 92	21
miR5200-3p	*LYST, TGFBR3*	–102 to –100	92 to 94	21
miR5384-3p	*MAF, PAX1*	–113 to –110	91 to 93	21
miR9652-5p	*LZTFL1, PTCHD1, STX6*	–100 to –98	90 to 92	22
miR9655-3p	*CIB2, GIPR, GPR123, GPR55, TACR1, ZNF488*	–102	91	21
miR9656-3p	*C9orf50*	–106	93	21
miR9661-5p	*DNAH14, GSE1, NUP50, SHANK1*	–104 to –102	91 to 92	21
miR9664-3p	*ORAOV1, SIK2*	–102	91	21
miR9667-5p	*REEP3*	–96	94	21
miR9670-3p	*TPM3, USP14*	–100 to –98	92 to 94	21
miR9772-5p	*C7*	–100	92	21
miR9773-3p	*CCDC178, CFLAR, INO80D, LRRC34, RHBDD1*	–100	90	24

Table 3 *(continued)*

Table 3 *(continued)*

tae-miRNA	Gene	ΔG, kJ/mol	ΔG/ΔGm, %	Length, nt
miR9774-3p	*NPFF*	–100	90	22
miR9776-5p	*SPRY4, TRAK2*	–106 to –102	91 to 94	21
miR9777-3p	*PDK2*	–93	92	20
miR9778-5p	*SORL1*	–102	91	21
miR9779-3p	*ASRGL1*	–100	96	20
miR9780-3p	*HES4, MEX3C, UBE2K*	–119 to –117	92 to 93	21
miR9781-3p	*LRP8, SLC38A2, PCLO, RNASE3*	–91 to –89	91 to 93	21
miR10520-5p	*TCFL5, TSPYL6*	–98 to –96	92 to 94	20

mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; nt, nucleotide.

Table 4 Characteristics of the interaction of tae-miRNA families with mRNAs of human target genes

tae-miRNA	Gene	ΔG, kJ/mol	ΔG/ΔGm, %	Length, nt
miR159a,b-3p	*KCNJ15, PKHD1*	–102	92	21
miR167a-c-5p	*ARHGEF10, EME1, HSF1, PRICKLE2*	–108 to –102	91 to 96	21
miR171b-3p	*LTB4R2*	–102	91	21
miR395a-3p	*CD22, EDN1, TEAD2*	–108 to –102	91 to 96	21
miR444a,b-3p	*AP1B1, COL6A3, DOCK1, HIC1, KRT6A, KRT6B, KRT6C, RHBDF1, RUNDC1*	–108 to –104	91 to 94	21
miR1120b-3p	*SEMA3A, FAM177A1*	–98	92	21
miR1120c-5p	*EPHA4*	–91	91	21
miR1122c-3p	*KCNAB3*	–100	92	21
miR1127b-3p	*SEMA6A*	–102	92	21
miR1137a-3p	*INO80D*	–96	96	20
miR9653a-3p	*ARHGEF6, CLCN7, ENDOD1, H1FOO, KDM6B, KIF7, PHLDB2*	–106 to –102	91 to 94	21
miR9653b-5p	*AOC3, ITIH4, RBM19, TEAD2, TEAD3, TMEM184A*	–113 to –104	91 to 98	21
miR9654b-3p	*NEBL*	–104	91	22
miR9657b-5p	*RNF168*	–104	92	21
miR9662a,b-3p	*DNMT3B, FAM124A, GLI2, HMX3*	–110 to –104	91	21
miR9666b-3p	*HMX3*	–110	91	22
miR9674b-5p	*ROBO3*	–102	92	21
miR9677a-3p	*AKT1, RBFOX3, STAC*	–108	91	22
miR9677b-5p	*ABCA3, CASKIN1, ENC1, GALR1, LRRFIP1, NLGN2, SNX8, SQSTM1*	–117 to –113	91 to 95	21

mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; nt, nucleotide.

Wheat miR444a,b-3p, which interacts with the CDS of nine mRNA genes, also had similar results (Table S10). Based on the nucleotides of the wheat miR444a,b-3p BSs (Table S11), the created WebLogo scheme demonstrates high conservation of some nucleotide sequences, which is identical at the 5- and 3-end of the BSs (*Figure 2*). Four G-C pairs with three hydrogen bonds each provide high free energy of interaction for wheat miR444a,b-3p with the mRNA of nine human genes. The characteristics of the interaction of wheat miR9653b-5p with the mRNA of seven human genes also show a high interaction efficiency (Table S12) and the conservation of flanking nucleotide sequences (Table S13 and *Figure 2*).

Discussion

The results show that many corn and wheat miRNAs have the potential to effectively interact with mRNA of human target genes. Similar studies done with rice miRNA are discussed in previous publication (87). Fully complementary interactions of plant miRNAs with mRNA genes have been previously established in many plant species, which may be important in the control of gene expression (80,82-84). Such an interaction between miRNAs and mRNAs has been already studied in animals (71,88,89). In the present work, the range of known examples was extended by studying the interaction between wheat and maize miRNAs and human mRNAs. Further studies can be done to determine which miRNAs of other plants can interact with mRNAs of human and animal genes.

The miRNAs are found in many plant tissues and organs' cells in relatively large quantities, which enables the determination of their functional importance Many plant miRNAs have the potential to regulate gene expression in a cross-kingdom manner (90-94). Along with juices, fruits, and vegetables a large number of plant miRNAs enter the human body (8).

The findings of *in silico* study contribute to the understanding of a potential function of plant miRNAs in the control of human gene expression. *In silico* analysis can be considered a first step for gene repression regulation analysis. There are obstacles and challenges that dietary miRNAs need to surpass to exert biological effects, including digestion. The literature describes that digestion can be an important part of possible function of miRNAs both in mammalian dietary miRNAs (95) and plant-derived miRNAs (96). Transport of dietary miRNAs within exosomes could help reduce the degradation of dietary miRNAs (97).

The interaction of plant miRNAs and human mRNA genes depend on many factors: for example, on the concentration of miRNAs in cells of various human organs, on the concentration of target genes in the cells, since a low concentration of plant miRNAs cannot significantly change the expression of the target gene. Both positive and negative effects of plant miRNAs may be shown on the human genome expression. For instance, some plant miRNAs can block the expression of oncogenes and suppress oncogenesis, while other plant miRNAs might promote tumor development by inhibiting oncosuppressors. As a result, in order to use plant miRNAs in medicine, extensive research needs to be done to understand the impact of these miRNAs on the function of various human genes. It will also allow to create synthetic analogs of plant miRNAs for therapeutic purposes (98).

Conclusions

Analysis of the possibilities of interaction of maize and wheat miRNAs with mRNAs of human target genes demonstrated that many of these plant miRNAs have potential to bind to human mRNA genes. zma-miRNAs and tae-miRNAs interacting with mRNA of human genes include the most well-known plant miRNAs important for the regulation of plant growth and development, as well as miRNAs that respond to plant stress. Many zma-miRNAs and tae-miRNAs can bind to mRNAs of target genes of various human diseases. Given the high conservation of some miRNAs in many plant species, one should expect edible plants to influence the expression of human genes. The results obtained in this work are consistent with the published data of our (72,74-76,80,82) and other researchers (8,20,21,50). Further study of such plant miRNAs may contribute to their medical applications.

Acknowledgments

Funding: None.

Footnote

Peer Review File: Available at https://exrna.amegroups.com/article/view/10.21037/exrna-23-4/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://exrna.

amegroups.com/article/view/10.21037/exrna-23-4/coif). PZ serves as an unpaid editorial board member of *ExRNA*. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Li Z, Xu R, Li N. MicroRNAs from plants to animals, do they define a new messenger for communication? Nutr Metab (Lond) 2018;15:68.
2. Samad AFA, Sajad M, Nazaruddin N, et al. MicroRNA and Transcription Factor: Key Players in Plant Regulatory Network. Front Plant Sci 2017;8:565.
3. Nair SK, Wang N, Turuspekov Y, et al. Cleistogamous flowering in barley arises from the suppression of microRNA-guided HvAP2 mRNA cleavage. Proc Natl Acad Sci U S A 2010;107:490-5.
4. Biswas K, Jolly MK, Ghosh A. First passage time properties of miRNA-mediated protein translation. J Theor Biol 2021;529:110863.
5. Sedaghat N, Fathy M, Modarressi MH, et al. Identifying functional cancer-specific miRNA-mRNA interactions in testicular germ cell tumor. J Theor Biol 2016;404:82-96.
6. Del Pozo-Acebo L, López de Las Hazas MC, Margollés A, et al. Eating microRNAs: pharmacological opportunities for cross-kingdom regulation and implications in host gene and gut microbiota modulation. Br J Pharmacol 2021;178:2218-45.
7. Liang G, Zhu Y, Sun B, et al. Assessing the survival of exogenous plant microRNA in mice. Food Sci Nutr 2014;2:380-8.
8. Liang H, Zhang S, Fu Z, et al. Effective detection and quantification of dietetically absorbed plant microRNAs in human plasma. J Nutr Biochem 2015;26:505-12.
9. Liu J, Wang F, Weng Z, et al. Soybean-derived miRNAs specifically inhibit proliferation and stimulate apoptosis of human colonic Caco-2 cancer cells but not normal mucosal cells in culture. Genomics 2020;112:2949-58.
10. Minutolo A, Potestà M, Gismondi A, et al. Olea europaea small RNA with functional homology to human miR34a in cross-kingdom interaction of anti-tumoral response. Sci Rep 2018;8:12413.
11. Minutolo A, Potestà M, Roglia V, et al. Plant microRNAs from Moringa oleifera Regulate Immune Response and HIV Infection. Front Pharmacol 2020;11:620038.
12. Potestà M, Roglia V, Fanelli M, et al. Effect of microvesicles from Moringa oleifera containing miRNA on proliferation and apoptosis in tumor cell lines. Cell Death Discov 2020;6:43.
13. Chen X, Rechavi O. Plant and animal small RNA communications between cells and organisms. Nat Rev Mol Cell Biol 2022;23:185-203.
14. Schuh CMAP, Cuenca J, Alcayaga-Miranda F, et al. Exosomes on the border of species and kingdom intercommunication. Transl Res 2019;210:80-98.
15. Chin AR, Fong MY, Somlo G, et al. Cross-kingdom inhibition of breast cancer growth by plant miR159. Cell Res 2016;26:217-28.
16. Zhang L, Hou D, Chen X, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 2012;22:107-26.
17. Roglia V, Potestà M, Minchella A, et al. Exogenous miRNAs from Moringa oleifera Lam. recover a dysregulated lipid metabolism. Front Mol Biosci 2022;9:1012359.
18. Hou D, He F, Ma L, et al. The potential atheroprotective role of plant MIR156a as a repressor of monocyte recruitment on inflamed human endothelial cells. J Nutr Biochem 2018;57:197-205.
19. Li M, Chen T, He JJ, et al. Plant MIR167e-5p Inhibits Enterocyte Proliferation by Targeting β-Catenin. Cells 2019;8:1385.
20. Li M, Chen T, Wang R, et al. Plant MIR156 regulates intestinal growth in mammals by targeting the Wnt/β-catenin pathway. Am J Physiol Cell Physiol 2019;317:C434-48.
21. Zempleni J, Baier SR, Howard KM, et al. Gene regulation by dietary microRNAs. Can J Physiol Pharmacol 2015;93:1097-102.
22. Cavalieri D, Rizzetto L, Tocci N, et al. Plant microRNAs as novel immunomodulatory agents. Sci Rep 2016;6:25761.

23. Samad AFA, Kamaroddin MF, Sajad M. Cross-Kingdom Regulation by Plant microRNAs Provides Novel Insight into Gene Regulation. Adv Nutr 2021;12:197-211.
24. Zhang L, Chen T, Yin Y, et al. Dietary microRNA-A Novel Functional Component of Food. Adv Nutr 2019;10:711-21.
25. Zhao Q, Liu Y, Zhang N, et al. Evidence for plant-derived xenomiRs based on a large-scale analysis of public small RNA sequencing data from human samples. PLoS One 2018;13:e0187519.
26. Marzano F, Caratozzolo MF, Consiglio A, et al. Plant miRNAs Reduce Cancer Cell Proliferation by Targeting MALAT1 and NEAT1: A Beneficial Cross-Kingdom Interaction. Front Genet 2020;11:552490.
27. He JJ, Ye R, Chen T, et al. Exploration of miRNAs in Neolamarckia cadamba and their potential cross-kingdom functions. ExRNA 2020;2:6.
28. Spinler JK, Oezguen N, Runge JK, et al. Dietary impact of a plant-derived microRNA on the gut microbiome. ExRNA 2020;2:11.
29. Jimenez-Jimenez S, Hashimoto K, Santana O, et al. Emerging roles of tetraspanins in plant inter-cellular and inter-kingdom communication. Plant Signal Behav 2019;14:e1581559.
30. Yang J, Farmer LM, Agyekum AA, et al. Detection of an Abundant Plant-Based Small RNA in Healthy Consumers. PLoS One 2015;10:e0137516.
31. Zhao C, Sun X, Li L. Biogenesis and function of extracellular miRNAs. ExRNA 2019;1:38.
32. Yarmarkovich M, Hirschi KD. Digesting dietary miRNA therapeutics. Oncotarget 2015;6:13848-9.
33. Melnik BC, Weiskirchen R, Schmitz G. Milk exosomal microRNAs: friend or foe?—a narrative review. ExRNA 2022;4:22.
34. Mutai E, Ramer-Tait AE, Zempleni J. MicroRNAs in bovine milk exosomes are bioavailable in humans but do not elicit a robust pro-inflammatory cytokine response. ExRNA 2020;2:2.
35. Li L, Wang J. Roles of extracellular microRNAs in central nervous system. ExRNA 2019;1:13.
36. Vickers KC, Palmisano BT, Shoucri BM, et al. MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. Nat Cell Biol 2011;13:423-33.
37. Turchinovich A, Weiz L, Langheinz A, et al. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223-33.
38. Iguchi H, Kosaka N, Ochiya T. Secretory microRNAs as a versatile communication tool. Commun Integr Biol 2010;3:478-81.
39. Witwer KW, Hirschi KD. Transfer and functional consequences of dietary microRNAs in vertebrates: concepts in search of corroboration: negative results challenge the hypothesis that dietary xenomiRs cross the gut and regulate genes in ingesting vertebrates, but important questions persist. Bioessays 2014;36:394-406.
40. Chen Q, Zhang F, Dong L, et al. SIDT1-dependent absorption in the stomach mediates host uptake of dietary and orally administered microRNAs. Cell Res 2021;31:247-58.
41. Witwer KW. XenomiRs and miRNA homeostasis in health and disease: evidence that diet and dietary miRNAs directly and indirectly influence circulating miRNA profiles. RNA Biol 2012;9:1147-54.
42. Luo Y, Wang P, Wang X, et al. Detection of dietetically absorbed maize-derived microRNAs in pigs. Sci Rep 2017;7:645.
43. Cui J, Zhou B, Ross SA, et al. Nutrition, microRNAs, and Human Health. Adv Nutr 2017;8:105-12.
44. Lukasik A, Zielenkiewicz P. Plant MicroRNAs-Novel Players in Natural Medicine? Int J Mol Sci 2016;18:9.
45. Kasarello K, Köhling I, Kosowska A, et al. The Anti-Inflammatory Effect of Cabbage Leaves Explained by the Influence of bol-miRNA172a on FAN Expression. Front Pharmacol 2022;13:846830.
46. Snow JW, Hale AE, Isaacs SK, et al. Ineffective delivery of diet-derived microRNAs to recipient animal organisms. RNA Biol 2013;10:1107-16.
47. Sun F, Guo G, Du J, et al. Whole-genome discovery of miRNAs and their targets in wheat (Triticum aestivum L.). BMC Plant Biol 2014;14:142.
48. Ding D, Wang Y, Han M, et al. MicroRNA transcriptomic analysis of heterosis during maize seed germination. PLoS One 2012;7:e39578.
49. Tian Y, Cai L, Tian Y, et al. miR156a Mimic Represses the Epithelial-Mesenchymal Transition of Human Nasopharyngeal Cancer Cells by Targeting Junctional Adhesion Molecule A. PLoS One 2016;11:e0157686.
50. Han R, Jian C, Lv J, et al. Identification and characterization of microRNAs in the flag leaf and developing seed of wheat (Triticum aestivum L.). BMC Genomics 2014;15:289.
51. Lukasik A, Pietrykowska H, Paczek L, et al. High-throughput sequencing identification of novel and conserved miRNAs in the Brassica oleracea leaves. BMC Genomics 2013;14:801.
52. Morea EG, da Silva EM, e Silva GF, et al. Functional and

evolutionary analyses of the miR156 and miR529 families in land plants. BMC Plant Biol 2016;16:40.
53. Yang T, Wang Y, Teotia S, et al. The interaction between miR160 and miR165/166 in the control of leaf development and drought tolerance in Arabidopsis. Sci Rep 2019;9:2832.
54. Pirrò S, Minutolo A, Galgani A, et al. Bioinformatics Prediction and Experimental Validation of MicroRNAs Involved in Cross-Kingdom Interaction. J Comput Biol 2016;23:976-89.
55. Dai X, Zhuang Z, Zhao PX. Computational analysis of miRNA targets in plants: current status and challenges. Brief Bioinform 2011;12:115-21.
56. Zhang B, Pan X, Wang Q, et al. Computational identification of microRNAs and their targets. Comput Biol Chem 2006;30:395-407.
57. Ergün S. Cross-Kingdom Gene regulation via miRNAs of Hypericum perforatum (St. John's wort) flower dietetically absorbed: An in silico approach to define potential biomarkers for prostate cancer. Comput Biol Chem 2019;80:16-22.
58. Singh N, Srivastava S, Shasany AK, et al. Identification of miRNAs and their targets involved in the secondary metabolic pathways of Mentha spp. Comput Biol Chem 2016;64:154-62.
59. Li J, Zhang Y. Current experimental strategies for intracellular target identification of microRNA. ExRNA 2019;1:6.
60. Tan ZL, Li JF, Luo HM, et al. Plant extracellular vesicles: A novel bioactive nanoparticle for tumor therapy. Front Pharmacol 2022;13:1006299.
61. Mohanty JN, Sahoo S, Routray SP, et al. Does the diverse source of miRNAs affect human health? An approach towards diagnosis and therapeutic management. Gene Rep 2022;28:101656.
62. Saiyed AN, Vasavada AR, Johar SRK. Recent trends in miRNA therapeutics and the application of plant miRNA for prevention and treatment of human diseases. Futur J Pharm Sci 2022;8:24.
63. Crooks GE, Hon G, Chandonia JM, et al. WebLogo: a sequence logo generator. Genome Res 2004;14:1188-90.
64. Schneider TD, Stephens RM. Sequence logos: a new way to display consensus sequences. Nucleic Acids Res 1990;18:6097-100.
65. Ivashchenko A, Berillo O, Pyrkova A, et al. MiR-3960 binding sites with mRNA of human genes. Bioinformation 2014;10:423-7.
66. Ivashchenko A, Pyrkova A, Niyazova R, et al. Prediction of miRNA binding sites in mRNA. Bioinformation 2016;12:237-40.
67. Garg A, Heinemann U. A novel form of RNA double helix based on G·U and C·A(+) wobble base pairing. RNA 2018;24:209-18.
68. Kool ET. Hydrogen bonding, base stacking, and steric effects in dna replication. Annu Rev Biophys Biomol Struct 2001;30:1-22.
69. Leontis NB, Stombaugh J, Westhof E. The non-Watson-Crick base pairs and their associated isostericity matrices. Nucleic Acids Res 2002;30:3497-531.
70. Lemieux S, Major F. RNA canonical and non-canonical base pairing types: a recognition method and complete repertoire. Nucleic Acids Res 2002;30:4250-63.
71. Yurikova OY, Aisina DE, Niyazova RE, et al. The Interaction of miRNA-5p and miRNA-3p with the mRNAs of Orthologous Genes. Mol Biol (Mosk) 2019;53:692-704.
72. Davis E, Caiment F, Tordoir X, et al. RNAi-mediated allelic trans-interaction at the imprinted Rtl1/Peg11 locus. Curr Biol 2005;15:743-9.
73. Koyama T, Sato F, Ohme-Takagi M. Roles of miR319 and TCP Transcription Factors in Leaf Development. Plant Physiol 2017;175:874-85.
74. Bonnet E, He Y, Billiau K, et al. TAPIR, a web server for the prediction of plant microRNA targets, including target mimics. Bioinformatics 2010;26:1566-8.
75. Dai X, Zhuang Z, Zhao PX. psRNATarget: a plant small RNA target analysis server (2017 release). Nucleic Acids Res 2018;46:W49-54.
76. Miranda KC, Huynh T, Tay Y, et al. A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 2006;126:1203-17.
77. Piñero J, Saüch J, Sanz F, et al. The DisGeNET cytoscape app: Exploring and visualizing disease genomics data. Comput Struct Biotechnol J 2021;19:2960-7.
78. Krämer A, Green J, Pollard J Jr, et al. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics 2014;30:523-30.
79. Ambros V, Bartel B, Bartel DP, et al. A uniform system for microRNA annotation. RNA 2003;9:277-9.
80. Bari A, Orazova S, Ivashchenko A. miR156- and miR171-binding sites in the protein-coding sequences of several plant genes. Biomed Res Int 2013;2013:307145.
81. Zhang LL, Li Y, Zheng YP, et al. Expressing a Target Mimic of miR156fhl-3p Enhances Rice Blast Disease Resistance Without Yield Penalty by Improving SPL14

Expression. Front Genet 2020;11:327.
82. Rakhmetullina A, Zielenkiewicz P, Pyrkova A, et al. Prediction of characteristics of interactions of miRNA with mRNA of GRAS, ERF, C2H2 genes of A. thaliana, O. sativa and Z. mays. Curr Plant Biol 2021;28:100224.
83. Rakhmetullina AK, Pyrkova AY, Goncharova AV, et al. Predicting characteristics of the potentially binding sites for miRNA in the mRNA of the TCP transcription factor genes of plants. Russ J Plant Physiol 2020;67:606-17.
84. Bari AA, Sagaidak AI, Pinskii IV, et al. Binding of miR396 to mRNA of genes encoding growth-regulating transcription factors of plants. Russ J Plant Physiol 2014;61:807-10.
85. Zhao Y, Xu K, Liu G, et al. Global identification and characterization of miRNA family members responsive to potassium deprivation in wheat (Triticum aestivum L.). Sci Rep 2020;10:15812.
86. Zhao XY, Hong P, Wu JY, et al. The tae-miR408-Mediated Control of TaTOC1 Genes Transcription Is Required for the Regulation of Heading Time in Wheat. Plant Physiol 2016;170:1578-94.
87. Rakhmetullina A, Pyrkova A, Aisina D, et al. In silico prediction of human genes as potential targets for rice miRNAs. Comput Biol Chem 2020. [Epub ahead of print]. doi: 10.1016/j.compbiolchem.2020.107305.
88. Atambayeva S, Niyazova R, Ivashchenko A, et al. The Binding Sites of miR-619-5p in the mRNAs of Human and Orthologous Genes. BMC Genomics 2017;18:428.
89. Myrzabekova M, Labeit S, Niyazova R, et al. Identification of Bovine miRNAs with the Potential to Affect Human Gene Expression. Front Genet 2021;12:705350.
90. Ito Y, Taniguchi K, Kuranaga Y, et al. Uptake of MicroRNAs from Exosome-Like Nanovesicles of Edible Plant Juice by Rat Enterocytes. Int J Mol Sci 2021;22:3749.
91. Li C, Wong AYP, Wang S, et al. miRNA-Mediated Interactions in and between Plants and Insects. Int J Mol Sci 2018;19:3239.
92. Ji H, Zhang K, Pan G, et al. Deoxyelephantopin Induces Apoptosis and Enhances Chemosensitivity of Colon Cancer via miR-205/Bcl2 Axis. Int J Mol Sci 2022;23:5051.
93. Woith E, Fuhrmann G, Melzig MF. Extracellular Vesicles-Connecting Kingdoms. Int J Mol Sci 2019;20:5695.
94. Lukasik A, Brzozowska I, Zielenkiewicz U, et al. Detection of Plant miRNAs Abundance in Human Breast Milk. Int J Mol Sci 2017;19:37.
95. López de Las Hazas MC, Del Pozo-Acebo L, Hansen MS, et al. Dietary bovine milk miRNAs transported in extracellular vesicles are partially stable during GI digestion, are bioavailable and reach target tissues but need a minimum dose to impact on gene expression. Eur J Nutr 2022;61:1043-56.
96. Chapado LA, Martín-Hernández R, Hernández de la Red S, et al. Connection between miRNA Mediation and the Bioactive Effects of Broccoli (Brassica oleracea var. italica): Exogenous miRNA Resistance to Food Processing and GI Digestion. J Agric Food Chem 2021;69:9326-37.
97. Del Pozo-Acebo L, López de Las Hazas MC, Tomé-Carneiro J, et al. Therapeutic potential of broccoli-derived extracellular vesicles as nanocarriers of exogenous miRNAs. Pharmacol Res 2022;185:106472.
98. Fu Y, Chen J, Huang Z. Recent progress in microRNA-based delivery systems for the treatment of human disease. ExRNA 2019;1:24.

doi: 10.21037/exrna-23-4
Cite this article as: Rakhmetullina A, Ivashchenko A, Pyrkova A, Uteulin K, Zielenkiewicz P. *In silico* analysis of maize and wheat miRNAs as potential regulators of human gene expression. ExRNA 2023;5:4.

Supplementary

Table S1 Comparative characteristics of programs that can be used to search for miRNA BSs in mRNA target genes

Programs	Schemes of interactions of miRNA with mRNA
MirTarget	5'-CUUCACCACCACCACCACCAC-3' 3'-AAAGUAGUAGUAGUAGUAGUA-5'
TAPIR	No BSs found
psRNATarget	No BSs found 21 AAAGUAGUAGUAGUAGUAGUA 1 886 UUUGAUCCUCAUCACCAUCAG 906
RNA 22	No BSs found TTTGATCCTCATCACCATCAG AAAGTAGTAGTAGTAGTAGTA 886

miRNA: ath-miR5658-5p; Gene: GSBRNA2T00009950001; Start of BSs: 610 nt., mRNA region: CDS, ΔG: –87 kJ/mol, ΔG/ΔGm: 85%, miRNA length: 21 nt. The predictions were compared with the results of psRNAtarget, Tapir, RNA22, with their default parameters. The default criteria (the score cutoff value =4 and the free energy ratio cutoff value =0.7) for target prediction in Tapir website were used. The standard parameter set for target prediction in psRNAtarget was as follows: penalty for (extending gap =0.5, opening gap =2, G.U pair =0.5, other mismatches =1), HSP size =19, seed region =2–13 nucleotides. The minimum expectation score =5.0. The RNA22 algorithm was run after defining standard settings. These include: Sensitivity and specificity values =63% and 61%, respectively, Seed size =7, with one unpaired base allowed in the seed region, Minimum number of paired-up bases in heteroduplex =12, Maximum folding energy for heteroduplex =–12 kcal/mol, Maximum number of G:U wobbles allowed in seed region = no limit. TAPIR, psRNATarget and RNA 22 analysis web servers did not find ath-miR5658-5p binding sites at positions 610 nt in the mRNA of the GSBRNA2T00009950001 gene, since the programs do not take into account hydrogen bonds between C and A. mRNA, messenger RNA; ath, *Arabidopsis thaliana*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BSs, binding sites; nt, nucleotide; CDS, coding domain sequence.

Table S2 Characteristics of zma-miR529-3p interaction with human mRNA genes

Gene	Start of BS, nt	Region of mRNA	ΔG, kJ/mol	ΔG/ΔGm, %
ATP6V0A4	241	5'UTR	–106	94
CHD2	6,684	3'UTR	–106	94
MLL	11,418	CDS	–106	94
PDE4B	2,270	CDS	–106	94
LMO7	4,210	CDS	–102	91
LRTOMT	1,111	CDS	–102	91
LTB4R	3,117	3'UTR	–102	91
NUDC	455	CDS	–102	91
POMC	1,055	CDS	–102	91
SOX9	92	5'UTR	–102	91

mRNA, messenger RNA; zma, *Zea mays*; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BS, binding sites; nt, nucleotide; CDS, coding domain sequence; 3'UTR, 3'-untranslated region; 5'UTR, 5'-untranslated region.

https://dx.doi.org/10.21037/exrna-23-4

Table S3 Nucleotide sequences of regions of human gene mRNA containing zma-miR529-3p BSs

Gene	Nucleotide sequences of mRNA regions
ATP6V0A4	UCUCUGAAGAGAAGAAGAGAGAGACACAGCCAAGACCGA
CHD2	UUGGGGUGGAGAAGUAGAGAGAGGGCAACAGCUUCCACAAC
MLL	CCCCAAUGAUGAAGAAGAGGAGGAGGUACAGCUGAAGUCAG
PDE4B	AAGGACCUGAGAAGGAGGGAGAGGGACACAGCUAUUUCAGC
LMO7	AACCCGAGCAGGAGAAGAGGAGAGGAGACAGCCACAAGAGG
LRTOMT	GGAACCCCAUGGAGGAAGAGAAAGGGUAUAGCAGGCCCUGG
LTB4R	GAGUGGAGUGGAAGAAGAGGGAGAGGUGGAGCAAAGUGAGG
NUDC	GCUAACUGAUGAAGAGGCAGAGAGGCUGCAGCUAGAGAUUG
POMC	ACGCCUACAAGAAGGGCGAGUGAGGGCACAGCGGGGCCCCA
SOX9	CUUUGCAGGAGGAGAAGAGAAGGGGUGCAAGCGCCCCCACU

The red type indicates nucleotide sequences of miRNA BSs. mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; BSs, binding sites.

Table S4 Characteristics of zma-miR159e-5p interaction with human mRNA genes

Gene	Start of BS, nt	Region of mRNA	ΔG, kJ/mol	ΔG/ΔGm, %
BZRAP1	72	5'UTR	–104	91
FEN1	1,040	CDS	–104	91
LZTS1	1,524	CDS	–106	93
PDE6B	218	CDS	–104	91
PRCD	755	3'UTR	–104	91

mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BS, binding sites; nt, nucleotide; CDS, coding domain sequence; 3'UTR, 3'-untranslated region; 5'UTR, 5'-untranslated region.

Table S5 Nucleotide sequences of regions of human gene mRNA containing zma-miR159e-5p BSs

Gene	Nucleotide sequences of mRNA regions
BZRAP1	GCAGUCCCCAGAGCAGAGGCCAGCAGGGGCUGAGACUAUGA
FEN1	AAUUCCACCUGAGCCGGAUUCUGCAGGAGCUGGGCCUGAAC
LZTS1	UCCUCGACGAGAGCAGAUGGCUGCAGCAGCUGCCAGACCUA
PDE6B	AGGUGGAGGAGAGCACGGCGCUGCUGGAGCUGGUGCAGGAU
PRCD	CCAGAUCCAGGAGCAGACCCUGCAGGCAGCUGCUCCUGAUG

The red type indicates nucleotide sequences of miRNA BSs. mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; BSs, binding sites.

https://dx.doi.org/10.21037/exrna-23-4

Table S6 Characteristics of zma-miR164b-3p interaction with human mRNA genes

Gene	Start of BS, nt	Region of mRNA	ΔG, kJ/mol	ΔG/ΔGm, %
DYSF	5,983	CDS	–100	92
EXOC7	2,252	CDS	–102	94
HPD	954	CDS	–100	92
ITGA7	3,496	CDS	–102	94
PTPRF	3,163	CDS	–100	92
ZNF37A	5,222	3'UTR	–102	94

mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BS, binding sites; nt, nucleotide; CDS, coding domain sequence; 3'UTR, 3'-untranslated region.

Table S7 Nucleotide sequences of regions of human gene mRNA containing zma-miR164b-3p BSs

Gene	Nucleotide sequences of mRNA regions
DYSF	UGAGCCUCACGGGGGAGAAGAUGAGCGACAUUUAUGUGAA
EXOC7	UCAAGUACGGGGUGGAGCAGGUGGGCGACAUGAUCGAUCG
HPD	CACACACCCUGGUGGAGAAGAUGAACUACAUCGGCCAAUU
ITGA7	AGCAGUUCAAGGAGGAGAAGACGGGCACCAUCCUGAGGAA
PTPRF	CGCCAGUGCUGGCGGAGAGGAACGGGCGCAUCAUCAGCUA
ZNF37A	GGAAGGGACAGGGGGAGGAGAUGGGCAGCAUUGUUGAGAG

The red type indicates nucleotide sequences of miRNA binding sites. mRNA, messenger RNA; zma, *Zea mays*; miRNA, mRNA-inhibitory RNA; BSs, binding sites.

Table S8 Characteristics of tae-miR408-3p interaction with human mRNA genes

Gene	Start of BS, nt	Region of mRNA	ΔG, kJ/mol	ΔG/ΔGm, %
RBMS2	7,343	3'UTR	–113	95
NGB	218	5'UTR	–108	91
ALPK3	602	CDS	–108	91
EDEM1	354	CDS	–108	91
PAQR6	1,079	CDS	–110	93
UNG	260	CDS	–108	91

mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BS, binding sites; nt, nucleotide; CDS, coding domain sequence; 3'UTR, 3'-untranslated region; 5'UTR, 5'-untranslated region.

https://dx.doi.org/10.21037/exrna-23-4

Table S9 Nucleotide sequences of regions of human gene mRNA containing tae-miR408-3p BSs

Gene	Nucleotide sequences of mRNA regions
RBMS2	GCUGUGAUAGGCCAGGGGAGUAGGCUGUGCAGUGACGGCUU
NGB	UCUCUCCCGCGCCAGGGAAGGAGCGGCUGCGGCCCCCGCCG
ALPK3	GUCGGGCCAGGCCAGGGGAGGGACAGCAGCAGGUGACGACG
EDEM1	GGCUGCAGCCGCCGGGGACCGGGGCAGCGCAGAGCCCGCGC
PAQR6	GCCUGGCCCGGCCCGGGAAGAGGCAGGGGCAGAUGCCUUCC
UNG	AGCUGCGGACGCCUGGGAAGGGGCCGCUGCAGCUCUUGAGC

The red type indicates nucleotide sequences of miRNA BSs. mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; BSs, binding sites.

Table S10 Characteristics of tae-miR444a,b-3p interaction in the CDS of human mRNA genes

Gene	Start of BS, nt	ΔG, kJ/mol	ΔG/ΔGm, %
AP1B1	2,787	−104	91
COL6A3	9,106	−104	91
DOCK1	1,960	−104	91
HIC1	2,077	−104	91
KRT6A	460	−104	91
KRT6B	299	−104	91
KRT6C	299	−104	91
RHBDF1	1,071	−108	94
RUNDC1	1,191	−108	94

mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BS, binding sites; nt, nucleotide; CDS, coding domain sequence.

Table S11 Nucleotide sequences of regions of human gene mRNA containing tae-miR444a,b-3p BSs

Gene	Nucleotide sequences of mRNA regions
AP1B1	GAGGCUGCGAGCAGCAAGCUGCAGAGCAGCAACAUCUUCAC
COL6A3	GCCAGCAACGGCAGCGAAGCCUGUAGCAGCAAAGCCAGCAG
DOCK1	CCAGCCUGCUGCAGCAGAACUUGAGGCAGCUGAUGAAAGUC
HIC1	UCACGGCCGAGCAGCUGAGCCUGAAGCAGCAGGACAAGGCG
KRT6A	GGCGGCUAUGGCAGCAGAGCCGGAGGCAGCUAUGGCUUUGG
KRT6B	GGCGGCUAUGGCAGCAGAGCCGGAGGCAGCUAUGGCUUUGG
KRT6C	GGCGGCUAUGGCAGCAGAGCCGGAGGCAGCUAUGGCUUUGG
RHBDF1	GCCCUGGACCGCAGCGAGCUUGAGCGCAGCCACCUGAUGCU
RUNDC1	ACAGAGUGAAGCAGCUAGCCUUGAGGCAGCAGCCACAUGAC

The red type indicates nucleotide sequences of miRNA BSs. mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; BSs, binding sites.

https://dx.doi.org/10.21037/exrna-23-4

Table S12 Characteristics of tae-miR9653b-5p interaction in the CDS of human mRNA genes

Gene	Start of BS, nt	Region of mRNA	ΔG, kJ/mol	ΔG/ΔGm, %
ITIH4	3,084	3'UTR	–106	93
TMEM184A	2,137	3'UTR	–104	91
AOC3	1,250	CDS	–113	98
LOC101927064	489	CDS	–104	91
RBM19	1,949	CDS	–104	91
TEAD2	245	CDS	–104	91
TEAD3	360	CDS	–104	91

mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; ΔG, free energy of miRNA binding; ΔGm, free energy of miRNA binding to a fully complementary nucleotide sequence; BSs, binding sites; nt, nucleotide; CDS, coding domain sequence; 3'UTR, 3'-untranslated region.

Table S13 Nucleotide sequences of regions of human gene mRNA containing tae-miR9653b-5p BSs

Gene	Nucleotide sequences of mRNA regions
ITIH4	UUCCACUGUCAGCUCUCAAGAGCCCAUGGCCAGGAAGGCCC
TMEM184A	UCCACACGCCAGCCACAGGGGAGCCCUGGCCAGGCGCCCAG
AOC3	UUAUGAGAUAAGCCUCCAAGAGGCCUUGGCCAUCUAUGGUG
LOC101927064	UCCACACGCCAGCCACAGGGGAGCCCUGGCCAGGCGCCCAG
RBM19	CUGGCGGCCCAGCUGCAGGAGACCUUCGGCCAUUUUGGCAG
TEAD2	CAUUGAGCAGAGCUUCCAGGAGGCCCUGGCCAUCUAUCCAC
TEAD3	CAUCGAGCAGAGCUUCCAGGAGGCCCUGGCCAUCUACCCGC

The red type indicates nucleotide sequences of miRNA BSs. mRNA, messenger RNA; tae, *Triticum aestivum*; miRNA, mRNA-inhibitory RNA; BSs, binding sites.

*Data in supplementary tables come in part from the PhD dissertation of AR: Characteristics of miRNAs binding with mRNAs of transcription factor genes of agricultural plants [dissertation]. Al-Farabi Kazakh National University; 2021

https://dx.doi.org/10.21037/exrna-23-4