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The characteristics of miRNA binding sites with mRNA of MYB plant transcription factors

Abstract: miRNA regulates the expression of a large number of genes, including plant transcription factors. It is important to reveal miRNA associations with the corresponding targets in order to increase plant productivity and resistance to biotic and abiotic stresses. One of such targets is the group of MYB transcription factor genes, involved in the regulation of some of the most important physiological processes. The search for miRNA target genes was performed using the MirTarget program, which calculates the free energy (ΔG) of miRNA binding with mRNA; position and schemes of the potential binding sites. The relative amount of free energy (ΔG/ΔGm) was used as a comparative criterion for evaluating the degree of interaction of miRNA and mRNA. The binding sites for tae-miR1127b-3p, tae-miR159a,b-3p, tae-miR164-5p, tae-miR171a-3p, tae-miR319-3p, tae-miR397-3p, tae-miR444a,b-3p, tae-miR5084-3p, tae-miR531-5p, tae-miR5384-3p, tae-miR9652-5p, tae-miR9663-5p, tae-miR9666a-3p, tae-miR9676-5p, tae-miR9778-5p, tae-miR9779-3p, tae-miR9780-3p were found in mRNAs of 258 MYB family genes in *Triticum (T.) aestivum*. miRNAs orthologs of *T. aestivum*, *Arabidopsis thaliana*, *Zea mays* and *Oryza sativa* had binding sites in mRNAs of MYB family genes of these and other plant species. tae-miR159a,b-3p binding sites are located in the CDS and encode the WSSIRSK oligopeptide, conserved in the proteins of the MYB transcription factors of 22 plant species. Amino acid sequences of the MYB family proteins containing ELPSNQ oligopeptide are encoded by tae-miR159a,b-3p binding sites located in the third open reading frame in the mRNAs of the other 20 plant species. For each miRNA, groups of target genes of transcription factors are established. The schemes of interaction of the nucleotide sequences of the studied miRNAs with the nucleotide sequences of the mRNA genes of the transcription factors of the MYB family were constructed. Considered associations of miRNAs and genes can be used as markers of control of plant physiological processes in plant growth selection and regulation.
Key words: miRNA, mRNA, gene, binding site, oligopeptide, plant, transcription factor, MYB.

Introduction

Regulation of gene expression on transcriptional level facilitates large number of important biological processes, including responses to different kinds of biotic and abiotic stresses, growth and development, differentiation, metabolism and other [1-5]. Transcription factors (TFs) play significant role in regulation of cellular processes. MYB proteins are among the most well-known, characterized by their highly conserved domains. A number of studies has shown that miRNAs can influence physiological processes in plants by miRNAs [6-10]. The established regulatory roles of miRNAs in gene expression unveil new opportunities to control plant productivity and resistance [11-14]. Nevertheless, in these works, no direct association of miRNAs with mRNAs of MYB transcription factors was not established. Therefore, the principal goal of our research was to establish miRNA associations and their MYB target genes using bioinformatic approaches, which in turn leads to significant reductions in material costs of search for such associations.

The MirTarget program that we created allows us to work with a large number of genes and miRNAs, predicting with high precision the associations of miRNAs and their target genes [15; 16].

In the present work, we focused on identification of miRNAs that have potential to bind to the mRNA genes of the MYB family in *Triticum (T.) aestivum*, *Oryza (O.) sativa, Arabidopsis (A.) thaliana and Zea (Z.) mays.*

Materials and methods

Nucleotide sequences of MYB genes of *T. aestivum, O. sativa, A. thaliana and Z. mays* were obtained from Plant Transcription Factor Database v.4.0 (http://planttfdb.cbi.pku.edu.cn/index.php; http://www.ncbi.nlm.nih.gov). Nucleotide sequences of miRNAs in genomes of studied plants were obtained from the database miRBase v.22 (http://www.mirbase.org/). The search for miRNA target genes was determined using the MirTarget program [15; 16]. It calculates the free energy (Δ*G*, kJ/mole) of miRNA binding, the relative value of free energy (ΔG/ΔGm, %), the position and schemes of potential binding sites (BSs). The ΔGm for miRNA binding was defined as the free energy of miRNA binding to the fully complementary nucleotide sequence. Relative amount of free energy (ΔG/ΔGm) was used as a comparative criterion to evaluate the degree of interaction of miRNA and mRNA. Unique properties of MirTarget program include consideration of nucleotide interaction in miRNA with mRNA of target genes not only between adenine (A) and uracil (U), guanine (G) and cytosine (C), but also between A and C, G and U via single hydrogen bond [17; 18]. The distance between A-C and G-U is equal to distance value between G-C and A-U.

List of abbreviated names of plant species of the following transcription factors were used in this work: *Aegilops tauschii – Ata, Aethionema arabicum – Aar, Arabidopsis halleri – Aha, Arabidopsis lyrata – Aly, Arabidopsis thaliana – Ath, Arabis alpina – Aal, Boechera stricta – Bst, Brachypodium distachyon – Bdi, Brachypodium stacei – Bsa, Brassica napus – Bna, Camelina sativa – Csa, Dichanthelium oligosanthes – Dol, Eragrostis tef – Ete, Leersia perrieri – Lpe, Musa acuminata – Mac, Oryza barthii – Oba, Oryza brachyantha – Obr, Oryza glaberrima – Ogl, Oryza glumaepatula – Ogu, Oryza longistaminata – Olo, Oryza meridionalis – Ome, Oryza nivara - Oni, Oryza punctata – Opu, Oryza rufipogon – Oru, Oryza sativa subsp. japonica – Osa j, Panicum hallii – Pha, Raphanus raphanistrum – Rra, Raphanus sativus – Rsa, Setaria italica – Sit, Setaria viridis – Svi, Sorghum bicolor – Sbi, Tarenaya hassleriana – Tha, Triticum aestivum – Tae, Zea mays – Zma.*

Results and discussion

Study of 125 miRNAs binding to mRNAs of 258 MYB family genes of *T. aestivum* revealed that only 34 genes were targets for 19 miRNA (Table 1).

Table 1 – Characteristics of miRNA BSs in CDS mRNA of MYB transcription factors genes of *T. aestivum*

Gene	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
Traes_2BL_79F1B50DF.1	tae-miR1127b-3p	706	-96	87	21
Traes_2AL_41B71F83C.1	tae-miR1127b-3p	691	-96	87	21
Traes_6DS_A0EC5D808.1	tae-miR159a,b-3p	688	-100	90	21
TRAES3BF027700010CFD_t1	tae-miR159a,b-3p	956	-98	88	21
Traes_2DL_912473A86.1	tae-miR159a,b-3p	54	-98	88	21
Traes_2BL_855A1170C.2	tae-miR159a,b-3p	141	-98	88	21
Traes_2AL_0A21FB42C.1	tae-miR159a,b-3p	141	-98	88	21
Traes_6AS_5562B97F7.1	tae-miR159a,b-3p	760	-96	87	21
Traes_1BL_CF98E922B.1	tae-miR159a,b-3p	839	-96	87	21
TRAES3BF034000040CFD_t1	tae-miR164-5p	629	-102	87	21
Traes_2BL_7CEC6A8D7.1	tae-miR164-5p	545	-102	87	21
Traes_2BL_03F3475CD.1	tae-miR164-5p	545	-102	87	21
Traes_2AL_AF9357B4C.1	tae-miR164-5p	548	-102	87	21
Traes_2AL_962A9D448.1	tae-miR164-5p	545	-102	87	21
Traes_1DS_5EEED86AD.2	tae-miR171a-3p	232	-96	87	21
Traes_1BS_403DBC53C.1	tae-miR171a-3p	577	-96	87	21
Traes_1AS_61D017632.2	tae-miR171a-3p	577	-96	87	21
TRAES3BF027700010CFD_t1	tae-miR319-3p	955	-102	89	21
Traes_1BL_CF98E922B.1	tae-miR319-3p	838	-102	89	21

Continuation of table 1

Gene	miRNA	Start of site, nt	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
Traes_4DS_7BFAC49C2.1	tae-miR397-3p	206	-104	89	21
TRAES3BF024100110CFD_t1	tae-miR444a,b-3p	707	-100	87	21
TRAES3BF012200030CFD_t1	tae-miR444a,b-3p	1151	-100	87	21
Traes_3B_934488D20.1	tae-miR444a,b-3p	1106	-100	87	21
Traes_3AL_152A7186A.1	tae-miR444a,b-3p	1052	-100	87	21
Traes_7DS_E27ECBC6E.1	tae-miR5084-3p	464	-106	86	24
Traes_7BS_D28CCE3B8.1	tae-miR5084-3p	413	-106	86	24
Traes_7AS_A337F362A.1	tae-miR5084-3p	413	-106	86	24
TRAES3BF026500080CFD_t1	tae-miR531-5p	157	-110	90	21
Traes_2DS_61B920833.1	tae-miR5384-3p	502	-108	89	21
TRAES3BF063000030CFD_t1	tae-miR9652-5p	214	-93	86	22
TRAES3BF026500080CFD_t1	tae-miR9663-5p	652	-96	87	21
Traes_2BL_361925B62.1	tae-miR9666a-3p	407	-106	88	21
Traes_7DL_5BD0D4BD1.1	tae-miR9676-5p	439	-104	87	22
Traes_7AL_04D939077.1	tae-miR9676-5p	439	-104	87	22
Traes_5BL_C1D4586B1.2	tae-miR9778-5p	431	-98	87	21
TRAES3BF012200020CFD_t1	tae-miR9779-3p	284	-96	92	20
Traes_2DS_3F5D36630.1	tae-miR9780-3p	958	-115	90	21

tae-miR1127b-3p, tae-miR159a,b-3p, tae-miR164-5p, tae-miR171a-3p, tae-miR319-3p, tae-miR397-3p, tae-miR444a,b-3p, tae-miR5084-3p, tae-miR531-5p, tae-miR5384-3p, tae-miR9652-5p, tae-miR9663-5p, tae-miR9666a-3p, tae-miR9676-5p, tae-miR9778-5p, tae-miR9779-3p, tae-miR9780-3p bind with mRNAs of these genes. For tae-miR159a, b-3p, there are 10 target genes with ΔG/ΔGm value ranging from 86 to 92%. tae-miR164-5p and tae-miR444a,b-3p had five and four target genes with ΔG/ ΔGm value of 87%. tae-miR171a-3p and tae-miR5084-3p bind to mRNAs of three MYB genes. ΔG/ΔGm value of interaction of tae-miR171a-3p and tae-miR5084-3p with mRNA of these genes was 87% and 86%, respectively. tae-miR1127b-3p, tae-miR319-3p tae-miR9676-5p had BSs in the mRNA of two target genes. The remaining miRNAs had only one target genes with a ΔG/ΔGm value ranging from 87 to 92%. The miRNA BSs in the mRNA genes of the MYB family of *T. aestivum* are located only in the coding sequence (CDS).

Not only MYB family genes of *T. aestivum*, but also genes of other plant species were targets for miRNAs. For instance, tae-miR159a,b-3p has binding sites in the mRNAs of *T. aestivum* Traes_2DL_912473A86.1, Traes_2BL_855A1170C.2, Traes_2AL_0A21FB42 C.1 genes as well as in *O. sativa* LOC_Os04g46384.1 gene, *A. thaliana* AT3G60460.1 gene, and *Z. mays* GRMZM2G311059_P01, GRMZM2G046443_P01 genes, which are the members of the MYB family of these plant species (Table 2).

Since the characteristics of miRNA159-3p interaction with mRNA genes of different plant species were close, here we provide data only for the miR159-3p family. The interaction patterns of the nucleotide sequences of miRNA with the mRNAs of these genes are presented in Figure 1.

It is important to note that the nucleotide sequence of tae-miR159a,b-3p is similar in the *T. aestivum*, *O. sativa, A. thaliana* and *Z. mays* genomes in which it was found (miRBase).

Six miRNAs of the zma-miR159a,c,d,f,j,k-3p family were bound to mRNAs of GRMZM2G311059_P01 and GRMZM2G046443_P01 genes with a ΔG/ΔGm value of 87 – 89%. Three members of ath-miR159a,b,c-3p family were bound to mRNA of AT3G60460.1 gene with a ΔG/ΔGm value of 86% and above. Two miRNAs of the osa-miR159a.1,f-3p and tae-miR159a,b-3p had two and three target genes, respectively (Table 2). The tae-miR159a,b-3p binding sites are located in the CDS and encode the WSSIRSK oligopeptide, which is conserved in the 27 proteins of the MYB transcription factors for 22 plant species (Table 3).

Table 2 – Characteristics of miR159-3p BSs in CDS mRNA of MYB transcription factors genes of *T. aestivum*, *O. sativa, A.thaliana*, *Z. mays*

Gene	miRNA	Start site, nt	ΔG, kJ/mole	ΔG/ΔGm, %
Traes_2DL_912473A86.1	tae-miR159a,b-3p	54	-98	88
Traes_2BL_855A1170C.2	tae-miR159a,b-3p	141	-98	88
Traes_2AL_0A21FB42C.1	tae-miR159a,b-3p	141	-98	88
LOC_Os04g46384.1	osa-miR159f-3p	199	-100	90
LOC_Os04g46384.1	osa-miR159a.1-3p	198	-98	88
AT3G60460.1	ath-miR159a-3p	182	-98	90
AT3G60460.1	ath-miR159c-3p	182	-98	88
AT3G60460.1	ath-miR159b-3p	182	-93	86
GRMZM2G311059_P01	zma-miR159a,f,j,k-3p	1393	-98	88
GRMZM2G311059_P01	zma-miR159c,d-3p	1394	-100	89
GRMZM2G046443_P01	zma-miR159c,d-3p	292	-100	89
GRMZM2G046443_P01	zma-miR159a,f,j,k-3p	292	-96	87

Gene,miRNA, site,region of mRNA, characteristics of binding	Gene,miRNA, site,region of mRNA, characteristics of binding
Traes_2DL_912473A86.1, tae-miR159b-3p, 55, -96, 87 5'-U**G**GAGCUCCAUUC**G**AUCCAAA-3' 3'-G**U**CUCGAGGGAAG**U**UAGGUUU-5'	AT3G60460.1, ath-miR159c-3p, 182, -98, 88 5'-UGGAGCUCCAUUC**G**AUCCAAA-3' 3'-UCCUCGAGGGAAG**U**UAGGUUU-5'
Traes_2BL_855A1170C.2, tae-miR159b-3p, 142, -66, 87 5'-U**G**GAGCUCCAUUCGAUCCAAA-3' 3'-G**U**CUCGAGGGAAGUUAGGUUU-5'	GRMZM2G311059_P01, zma-miR159a-3p, 1394, -96, 87 5'-**UG**GAGCUCCAUUC**G**AUCCAAA-3' 3'-**GU**CUCGAGGGAAG**U**UAGGUUU-5'
LOC_Os04g46384.1, osa-miR159a.1-3p, 198, -98, 88 5'-CUGGAGCUCCAUUC**G**AUCCAAA-3' 3'-GUC-UCGAGGGAAG**U**UAGGUUU-5'	GRMZM2G311059_P01, zma-miR159f-3p, 1394, -96, 87 5'-**UG**GAGCUCCAUUC**G**AUCCAAA-3' 3'-**GU**CUCGAGGGAAG**U**UAGGUUU-5'
LOC_Os04g46384.1, osa-miR159f-3p, 199, -100, 90 5'-U**G**GAGCUCCAUUC**G**AUCCAAAG-3' 3'-A**U**CUCGAGGGAAG**U**UAGGU-UC-5'	GRMZM2G311059_P01, zma-miR159j-3p, 1394, -96, 87 5'-**UG**GAGCUCCAUUC**G**AUCCAAA-3' 3'-**GU**CUCGAGGGAAG**U**UAGGUUU-5'
AT3G60460.1, ath-miR159a-3p, 182, -98, 90 5'-U**G**GAGCUCCAUUC**G**AUCCAAA-3' 3'-A**U**CUCGAGGGAAG**U**UAGGUUU-5'	GRMZM2G046443_P01, zma-miR159a-3p, 292, -96, 87 5'-**UG**GAGCUCCAUUC**G**AUCCAAA-3' 3'-**GU**CUCGAGGGAAG**U**UAGGUUU-5'
AT3G60460.1, ath-miR159b-3p, 182, -93, 86 5'-U**G**GAGCUCCAUUC**G**AUCCAAA-3' 3'-U**U**CUCGAGGGAAG**U**UAGGUUU-5'	GRMZM2G046443_P01, zma-miR159j-3p, 292, -96, 87 5'-**UG**GAGCUCCAUUC**G**AUCCAAA-3' 3'-**GU**CUCGAGGGAAG**U**UAGGUUU-5'
The bold type indicates the nucleotide of non-canonical pairs U-G, A-C.	

Figure 1 – Schemes of miR159-3p interaction with mRNAs of MYB transcription factor genes in *T. aestivum*, *O. sativa, A.thaliana*, *Z. mays*

Table 3 – The variability of amino acid sequences of the MYB family proteins containing oligopeptide WSSIRSK encoded by the binding sites of miR159-3p in the mRNA of genes

Gene	Abbreviated names	Region of transcription factor containing oligopeptide WSSIRSK
GRMZM2G311059_P01	*Zma*	LLRHVLVHGPRD**WSSIRSK**GFLPRTGKSCRL
GRMZM2G046443_P01	*Zma*	LRRHVMENGPRE**WSSIRSK**GLLPRTGKSCRL
LOC_Os04g46384.1	*Osaj*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
Traes_2DL_912473A86.1	*Tae*	LLEHVRTHGPRD**WSSIRSK**GALQRTGKSCRL
Traes_2BL_855A1170C.2	*Tae*	LLEHVRTHGPRD**WSSIRSK**GALQRTGKSCRL
Traes_2DL_912473A86.1	*Tae*	LLEHVRTHGPRD**WSSIRSK**GALQRTGKSCRL
Traes_2BL_855A1170C.2	*Tae*	LLEHVRTHGPRD**WSSIRSK**GALQRTGKSCRL
Traes_2AL_0A21FB42C.1	*Tae*	LLEHVRTHGPRD**WSSIRSK**GALQRTGKSCRL
AT3G60460.1	*Ath*	LINHVKRYGPRD**WSSIRSK**GLLQRTGKSCRL
Bradi5g17600.2.p	*Bdi*	LLEHVRTHGPCD**WSSIRSK**GILPRTGKSCRL
Brast09G163900.1.p	*Bsa*	LLEHVRAHGPCD**WSSIRSK**GILPRTGKSCRL
Do012459.1	*Dol*	LLEHVRAHGPCD**WSSIRSK**GLLPRTGKSCRL
462873087	*Ete*	LREHVRTHGPRD**WSSIRSK**GLLPRTGKSCRL
LPERR04G16870.1	*Lpe*	LREHVRTHGPRE**WSSIRSK**VGLPRTGKSCRL
GSMUA_Achr1P01660_001	*Mac*	LMEYVRKHGPRD**WSSIRSK**GLLARTGKSCRL
OBART04G20790.1	*Oba*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
OB04G27810.1	*Obr*	LLQHVRAHGPMD**WSSIRSK**GLLPRTGKSCRL
ORGLA04G0178400.1	*Ogl*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
OGLUM04G20730.1	*Ogu*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
KN540032.1_FGP006	*Olo*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
OMERI04G17240.1	*Ome*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
OPUNC04G18480.1	*Opu*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
ORUFI04G22380.1	*Oru*	LLEHVRTHGPMD**WSSIRSK**GLLPRTGKSCRL
Pahal.F00780.1	*Pha*	LLRHVREHGPRE**WSSIRSK**GLLPRTGKSCRL
Sobic.006G169700.1.p	*Sbi*	LLEHVRVHGPRD**WSSIRSK**GFLPRTGKSCRL
Seita.6G211500.1.p	*Sit*	LLRHVREHGPRE**WSSIRSK**GLLPRTGKSCRL
Sevir.6G218900.1.p	*Svi*	LLRHVREHGPRE**WSSIRSK**GLLPRTGKSCRL

The zma-miR159c,d,e,f,j,k-3p family consists of zma-miR159c,d-3p, zma-miR159e-3p and zma-miR159f,j,k-3p different in 5’ and 3’ ends of nucleotide sequences. Therefore, their binding sites are identical. Table 4 shows the binding characteristics of mir159-3p with the mRNA of MYB family genes. All sites are located in the CDS of the mRNA target. The binding characteristics of miR159-3p in the mRNAs CDS of Traes_6DS_A0EC5D808.1, AT2G32460.1, AC217264.3_FGP005, GRMZM2G070523_P01 genes are shown on Figure 2.

The nucleotide sequences of miR159-3p interacted along the entire length with the corresponding mRNA. Data from the analysis of miR159-3p binding to the mRNA of 23 genes in 20 plant species are listed in Table 5.

Table 4 – Characteristics of miR159-3p BSs in CDS mRNA of MYB transcription factors genes of *T. aestivum*, *O. sativa, A.thaliana*, *Z. mays*

Gene	miRNA	Start site, nt	ΔG, kJ/mole	ΔG/ΔGm, %
AT2G32460.1	ath-miR159a-3p	1016	-100	92
AT2G32460.1	ath-miR159b-3p	1016	-96	88
LOC_Os06g46560.1	osa-miR159d-3p	1064	-100	89
Traes_6DS_A0EC5D808.1	tae-miR159a,b-3p	688	-100	90
Traes_6AS_5562B97F7.1	tae-miR159a,b-3p	760	-96	87
AC217264.3_FGP005	zma-miR159a-3p	1377	-96	87
GRMZM2G070523_P01	zma-miR159a-3p	1044	-96	87
AC217264.3_FGP005	zma-miR159c,d-3p	1377	-98	87
GRMZM2G070523_P01	zma-miR159c,d-3p	1044	-98	87
AC217264.3_FGP005	zma-miR159e-3p	1377	-110	100
GRMZM2G070523_P01	zma-miR159e-3p	1044	-110	100
AC217264.3_FGP005	zma-miR159f,j,k-3p	1377	-96	87
GRMZM2G070523_P01	zma-miR159f,j,k-3p	1044	-96	87

Gene, miRNA, site, region of mRNA, characteristics of binding	Gene, miRNA, site, region of mRNA, characteristics of binding
Traes_6DS_A0EC5D808.1, tae-miR159a-3p, 688, -100, 90 5'–CAGGAGCUCCCUUC**G**AACCAAU–3' 3'–GUC–UCGAGGGAAG**U**UAGGUUU–5'	AC217264.3_FGP005, zma-miR159c-3p, 1377, -110, 100 5'–UGGAGCUCCCUUCAAACCAAU–3' 3'–ACCUCGAGGGAAGUUUGGUUA–5'
AT2G32460.1, ath-miR159a-3p, 1016, 100, 92 5'–UAGAGCU**U**CCUUCAAACCAAA–3' 3'–AUCUCGA**G**GGAAGUUAGGUUU–5'	GRMZM2G070523_P01, zma-miR159c-3p, 1044, -110, 100 5'–UGGAGCUCCCUUCAAACCAAU–3' 3'–ACCUCGAGGGAAGUUUGGUUA–5'
The bold type indicates the nucleotide of non-canonical pairs U-G, A-C.	

Figure 2 – Schemes of miR159-3p BSs interaction in CDS of *T. aestivum*, *A.thaliana, Z. mays* MYB transcription factor genes mRNAs

Table 5 – The variability of amino acid sequences of the MYB family proteins containing oligopeptide ELPSNQ encoded by the binding sites of miR159-3p in the mRNA of different plant genes

Gene	Abreviated names	Region of transcription factor containing oligopeptide ELPSNQ
AC217264.3_FGP005	*Zma*	LPPLSPSPGPRV**ELPSNQ**YGQPAPPTSAAA
GRMZM2G070523_P01	*Zma*	PPLSPSPCPRVV**ELPSNQ**YAQPTPPASAAA
AT2G32460.1	*Ath*	SSFPLGLDNSVL**ELPSNQ**RPTHSFSSSPII
Traes_6DS_A0EC5D808.1	*Tae*	PGMPPLVPPAVQ**ELPSNQ**SPADAGGPLEML
Traes_6AS_5562B97F7.1	*Tae*	LGMPPLVPPSAQ**ELPSNQ**SPADAGGPLEML
LOC_Os06g46560.1	*Osaj*	SSGLPPLPNRPR**ELPSNQ**FETATSGGGGGC
KFK31122.1	*Aal*	GMIKPTSFPLGL**ELPSNQ**TPTHSFTSNTIL
AA32G00526	*Aar*	NITPNSFLLQNL**ELPSNQ**TTSNTNKNGVFL
Araha.1971s0002.1.p	*Aha*	SSFPLGLENSVL**ELPSNQ**TPTHSFSSNPIL
482173	*Aly*	SSFPLGLENSVL**ELPSNQ**TPTHSFSSNPIL

Continuation of table 5

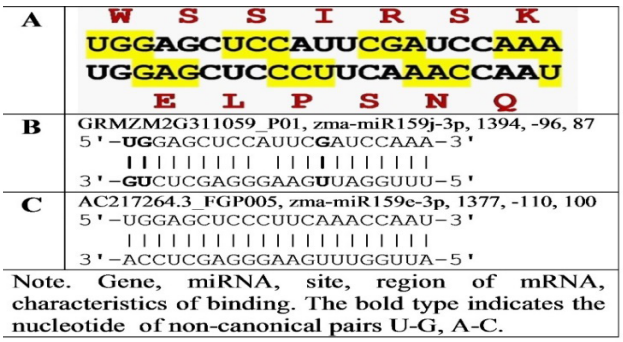
GSBRNA2T00006425001	*Bna*	SYFSLGLDTTVL**ELPSNQ**TPCTSNIMHDNN
XP_013634909.1	*Tha*	SYFSLGLDTTVL**ELPSNQ**TPTQSCTSNIML
Csa05g024410.1	*Csa*	SSFPLGLENTVL**ELPSNQ**TTIDSFTSNPIL
Bostr.23794s0867.1.p	*Bst*	SSFPLGLGNTVL**ELPSNQ**TPTHSFTSNPIL
RrC14648_p1	*Rra*	SYFSLGLDNTVL**ELPSNQ**TPTQLCTSNIML
Rsa1.0_01027.1_g00010.1	*Rsa*	SYFSLGLDNTVL**ELPSNQ**TPTQLCTSNIML
ORUFI06G26670.1	*Oru*	HAXLPPLPNRPR**ELPSNQ**FETATSGGGGGC
ONIVA06G28020.1	*Oni*	SSGLPPLPNRPR**ELPSNQ**FETATSGGGGGG
OMERI06G24960.1	*Ome*	SPSASQANSPPR**ELPSNQ**FETATSGGGGGD
OGLUM06G26120.1	*Ogu*	SSGLPPLPNRPR**ELPSNQ**FETATSGGGGGG
Do015678.1	*Dol*	YSGLPPLPTRPQ**ELPSNQ**FDTSSSGGGGAG
EMT12896	*Ata*	LPGLPPLPTRPR**ELPSNQ**IETASCSGGADG
EMT06644	*Ata*	PGMPPLVPPAVQ**ELPSNQ**SPADAGGPLEML

This data indicates that the relationship between miR159-3p and mRNA of target genes arose millions of years ago, which suggests its important functional significance.

The nucleotides of miR159-3p binding sites are homologous, but encode different oligopeptides (Figure 3 A, B).

Figure 3 – Variability of amino acid region of MYB family proteins on the example of tae-miR159a,b-3p binding sites. Note: **(A)** – oligopeptide WSSIRSK encoded by tae-miR159a,b-3p binding sites in mRNAs of different plant species; **(B)** – oligopeptide ELPSNQ encoded by tae-miR159a,b-3p binding sites located in the third open reading frame in the mRNAs of other plant species

The data in Figure 3 is explained by the fact that mRNA nucleotide sequences can encode different oligopeptides in different reading frames. Therefore, miRNA binding sites in mRNA can also encode different oligopeptides. The binding sites of some miRNAs have homologous nucleotide sequences that can be read in different open reading frames. For example, the nucleotide sequence of miR159j-3p binding sites UGGAGCUCCAUUCGAUCCAAA in the first reading frame will encode the WSSIRSK oligopeptide, and in the third reading frame, miR159e-3p will encode the ELPSNQ oligopeptide (Figure 4).



Note. Gene, miRNA, site, region of mRNA, characteristics of binding. The bold type indicates the nucleotide of non-canonical pairs U-G, A-C.

Figure 4 – Scheme of zma-miR159j-3p and zma-miR159e-3p interaction with mRNAs of GRMZM2G311059_P01 and AC217264.3_FGP005 genes. Note: **A** – WSSIRSK and ELPSNQ oligopeptide coding scheme by zma-miR159j-3p and zma-miR159e-3p binding sites, respectively (yellow color indicates codons in different open reading frames); **B** – scheme of zma-miR159j-3p interaction with mRNA of GRMZM2G311059_P01gene; **C** – scheme of zma-miR159e-3p interaction with mRNA of AC217264.3_FGP005 gene.

Thus, on the example of interaction of zma-miR159j-3p and zma-miR159e-3p, having homologous nucleotide sequences, with mRNAs of the transcription factor genes of numerous plants, miRNAs binding is observed independently of the reading frame in which mRNA nucleotide sequence is translated. Similar data was obtained when analyzing the binding sites of miRNAs in animal mRNAs [19; 20].

Conclusion

Current work has shown that miRNA families can interact with mRNAs of MYB transcription fac-

tor genes and regulate their expression. In mRNAs of MYB genes of *Triticum aestivum*, *Oryza sativa, Arabidopsis thaliana*, *Zea mays* all binding sites to mir159-3p were located in protein coding part. These binding sites were homologous and encoded oligopeptides WSSIRSK and ELPSNQ. It is important to note that miRNA binding to CDS of the mRNAs encoded by the transcription factor genes is not accidental. Such localization of miRNA binding sites in mRNAs indicates conserved relationship for a many millions of years of divergence in the studied plant species. Analysis of interactions of miR159-3p with the different plants of MYB indicates the conserved structure of miRNA binding sites. A high ΔG/ΔGm ratio of miRNAs binding to miRNAs shows that the expression of genes of MYB family can be suppressed strongly by miR159-3p. 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.

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