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CHARACTERISTICS OF INTERACTION OF MIRNAS WITH MRNA GENES OF HEAT STRESS TRANSCRIPTION FACTORS

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Heat stress transcription factors (HSF) play a key role in plants response to several abiotic stresses by controlling the expression of stress-responsive genes, such as heat shock proteins [1, 2]. In recent years, the important role of miRNAs in regulating the expression of many plant genes has been established [3, 4]. However limited studies has been reported for the association of miRNA with the expression of plant HSF genes. In the present work, the task is to determine the characteristics of the interaction of miRNA with the mRNA genes of the HSF family *Arabidopsis thaliana*, *Oryza sativa*, *Triticum aestivum*, *Zea mays*. The nucleotide mRNA sequences of the HSF family were taken from Plant Transcription Factor Database v4.0 (<http://planttfdb.cbi.pku.edu.cn/index.php>; <https://www.ncbi.nlm.nih.gov/>). miRNAs were borrowed from miRBase v. 22 (<http://www.mirbase.org/>). The search for miRNA target genes was carried out in the MirTarget program [5, 6]. This program defines the start of miRNA binding sites with mRNA; the localization of miRNA binding sites in the 5' – untranslated region (5'UTR), the protein-coding region (CDS) and the 3' – untranslated region (3'UTR) of mRNA; the free energy of hybridization (ΔG , kJ/mole) and schemes of nucleotide interactions between miRNA and mRNA. The miRNA binding sites were selected to have the $\Delta G/\Delta G_m$ ratio of more than 85 %, taking into account the ΔG value and miRNA length.

Table 1 shows the characteristics of the miRNA binding sites in the mRNA of the HSF genes of *A. thaliana*, *O. sativa*, *T. aestivum*, *Z. mays*. *A. thaliana* is a model plant organism for modern molecular research and we found binding sites between miRNA and mRNA of their target genes. The binding of 428 ath-miRNAs to mRNA of 24 HSF genes of *A. thaliana* was studied. Only 14 genes were targets for 18 miRNAs. miR5021-5p and miR5658-5p had three target genes with a $\Delta G/\Delta G_m$ value from 85 % to 91 %. Two miRNAs of the miR172c, d-3p family, were bound with mRNA of the *AT5G03720.1* gene. The remaining miRNAs had only one target genes. The miRNA binding sites were located in 5'UTR, CDS and 3'UTR mRNA HSF genes of *A. thaliana*.

Table 1 – Characteristics of miRNA binding sites in mRNA of *A. thaliana*, *O. sativa*, *T. aestivum*, *Z. mays* HSF genes

Gene	miRNA	Start of site, nt	Region	ΔG , kJ/mole	$\Delta G/\Delta G_m$, %	Length, nt
Arabidopsis thaliana						
AT4G11660.1	miR5021-5p	215	5'UTR	-89	91	20
AT4G18880.1	miR415-5p	752	5'UTR	-96	90	21
AT4G17750.1	miR5631-3p	728	CDS	-93	90	21
AT4G18870.1	miR827-3p	877	CDS	-93	90	21
AT1G46264.1	miR834-3p	653	CDS	-100	89	21
AT5G16820.1	miR4245-3p	591	CDS	-87	87	21
AT1G67970.1	miR5021-5p	227÷230	CDS	-85	87	20
AT3G63350.1	miR5021-5p	590	CDS	-85	87	20
AT5G03720.1	miR5658-5p	1067	CDS	-89	87	21
Oryza sativa						
LOC_Os06g35960.1	miR5075-3p	547	CDS	-113	91	21
LOC_Os06g36930.1	miR5077-5p	486	CDS	-96	90	19
LOC_Os10g28340.1	miR529a-3p	47	5'UTR	-96	90	20
LOC_Os08g36700.1	miR5075-3p	974	CDS	-110	90	21
LOC_Os01g53220.1	miR5809-3p	233	CDS	-104	89	20
LOC_Os08g43334.1	miR2907a, b, c, d-3p	987	CDS	-117	89	22
LOC_Os09g28200.1	miR2925-5p	1071	CDS	-100	89	19
LOC_Os02g29340.1	miR164d-5p	76	5'UTR	-102	87	21
LOC_Os09g28354.1	miR169d-5p	1053	CDS	-98	87	21
LOC_Os10g28340.1	miR1862f, g-3p	758	CDS	-87	87	20
LOC_Os05g45410.1	miR2097-3p	896	CDS	-98	87	22
LOC_Os03g12370.1	miR396e-5p	1042	CDS	-96	87	21
LOC_Os03g63750.1	miR530-3p	538	CDS	-100	87	21
LOC_Os01g43590.1	miR2102-5p	616	CDS	-106	88	20
LOC_Os03g53340.1	miR2102-5p	961	CDS	-106	88	20
LOC_Os06g36930.1	miR2102-5p	395	CDS	-106	88	20
LOC_Os09g35790.1	miR2102-5p	937	CDS	-106	88	20
LOC_Os03g53340.1	miR414-5p	1525	CDS	-98	88	21
LOC_Os07g44690.1	miR5075-3p	302	CDS	-108	88	21
LOC_Os08g43334.1	miR5075-3p	1397	CDS	-108	88	21
LOC_Os09g28200.1	miR5075-3p	292	CDS	-108	88	21
LOC_Os09g28354.1	miR5075-3p	199	5'UTR	-108	88	21
LOC_Os09g35790.1	miR5075-3p	1410	CDS	-108	88	21
Triticum aestivum						
TRAES3BF071100100CFD t1	miR9778-5p	39	CDS	-100	89	21
TRAES3BF021000010CFD t1	miR9676-5p	41	CDS	-104	87	22
Traes 4BL B64C157DC.1	miR9657b, c-3p	651	CDS	-102	87	21
Zea mays						
GRMZM2G301485 P01	miR164a-3p	488	CDS	-102	91	21
GRMZM2G118453 P01	miR164a-3p	215	5'UTR	-100	89	21
GRMZM2G089525 P01	miR172c-5p	1181	CDS	-93	88	20
GRMZM2G179802 P01	miR164d-3p	1214	CDS	-96	88	20
GRMZM2G002131 P01	miR169c, r-5p	1102	CDS	-102	87	21
GRMZM2G173090 P01	miR164f-3p	687	CDS	-102	87	21
GRMZM2G173090 P01	miR167d-3p	1473	3'UTR	-110	87	23

The quantitative characteristics of 738 osa-miRNAs binding to mRNA of 25 HSF family genes of *O. sativa* were established. 21 genes were under the control of miRNA. 47 miRNAs were bound to mRNAs HSF, of which the largest number of binding sites had miR5075-3p. It bound to mRNA of nine genes (*LOC_Os04g48030.1*, *LOC_Os05g45410.1*, *LOC_Os06g35960.1*, *LOC_Os07g44690.1*, *LOC_Os08g36700.1*, *LOC_Os08g43334.1*, *LOC_Os09g28200.1*, *LOC_Os09g28354.1*, *LOC_Os09g35790.1*) with free energy from -106 kJ/mole to -113 kJ/mole and $\Delta G/\Delta G_m$ value from 86 % to 90 %. miR2102-5p had binding sites

in mRNA of six genes with $\Delta G/\Delta G_m$ value from 86 % to 88 %. miR5809–3p interacted with mRNA of three genes (*LOC Os01g53220.1*, *LOC Os06g35960.1*, *LOC Os07g44690*) with a $\Delta G/\Delta G_m$ value of 85 %-89 %. Two miRNA of the miR1846a, b-3p family, were bound with mRNA of *LOC Os04g48030.1*, *LOC Os08g43334.1*, *LOC Os09g28200.1*, *LOC Os09g28354.1* genes. Each miRNA of miR1428e, f, g-5p, miR169b, c, d-5p, miR1862f, g-3p, miR2907a, b, d-3p, c-5p, miR5539a, b-5p, miR814a, b, c-5p had one binding site in the mRNA of the *LOC Os09g35790.1*, *LOC Os09g28354.1*, *LOC Os10g28340.1*, *LOC Os08g43334.1*, *LOC Os05g45410.1*, *LOC Os03g63750.1* genes, respectively. miR529a-3p, miR530-3p, miR535-5p, miR5819-5p, miR6249a, b-5p had two binding sites in the mRNA of the HSF genes. miR1435-5p, miR164d-5p, miR2091-5p, miR2097-3p, miR2863b-3p, miR2866-5p, miR2870-3p, miR2924-3p, miR2925-5p, miR396e-5p, miR3982-5p, miR414-5p, miR5076-3p, miR5077-5p, miR5338-5p, miR5530-5p, miR5544-3p, miR5820-3p, miR5837.2-5p had only one target gene. The miRNA binding sites were located in 5'UTR and CDS mRNA HSF family genes of *O. sativa*.

To identify miRNAs that are targets genes of the HSF transcription factor family, a search was made for the binding sites of 125 taе-miRNAs with the mRNA of 51 genes of the *T. aestivum* HSF family. As a result of the search, it was found that six genes of the HSF family were targets of eight miRNAs.

miR5384-3p, miR9654b-3p, miR9657a, b, c-3p, miR9670-3p, miR9676-5p, miR9778-5p interacted with the mRNA of these genes. miR9654b-3p had binding sites in the mRNA of *Traes_5AL_16AD8DEEC.1*, *Traes_5BL_8D23DFA4A.1* genes. Three miRNAs of miR9657a, b, c-3p family, were bound with mRNA of the *Traes_4BL_B64C157DC.1* gene. miR5384-3p and miR9676-5p interacted with mRNA *TRAES3BF021000010CFD_t1* gene. One miRNA was bound with mRNA of *Traes_1AL_A4B5C1474.2* and *TRAES3BF071100100CFD_t1* genes. All discovered miRNA binding sites in mRNA of HSF family genes of *T. aestivum* were located only in the CDS. In the absence of complete complementarity of the interaction of miRNA with mRNA of genes, unpaired nucleotides appear, which leads to decrease of free energy of interaction of miRNA and mRNA.

As a result of studying the binding of 325 zma-miRNAs to mRNA 28 HSF family genes of *Z. mays* it was revealed that only only 13 targets targets for 20 miRNA. With mRNA of target genes miR156j, l-3p, miR156k-5p, miR164a, d, f-3p, miR166k-5p, miR167d, g-3p, miR169a, b, c, p, r-5p, miR172b, c, d-5p, miR444a, b-3p, miR482-3p were interacted. The miR164a, d, f-3p family had the largest number of target genes (four). They bound to the mRNA of *GRMZM2G118453_P01*, *GRMZM2G301485_P01*, *GRMZM2G179802_P01*, *GRMZM2G173090_P01* genes with free energy of -96 kJ/mole to -102 kJ/mole and $\Delta G/\Delta G_m$ value from 87 % to 91 %. miR167d, g-3p interacted with the mRNA of three genes: *GRMZM2G173090_P01*, *GRMZM2G089525_P01* and *GRMZM2G164909_P01*. In the mRNA of the *GRMZM2G002131_P01* gene, binding sites for miR156j, l-3p and miR169a, b, c, p, r-5p were found. miR482-3p bound with the mRNA of two target genes – *GRMZM2G086880_P01* and *GRMZM2G115456_P01*. miR444a, b-3p, miR172b, c, d-5p, miR166k-5p had one target gene. The miRNA binding sites were located in 5'UTR, CDS and 3'UTR mRNA HSF genes of *Z. mays*.

In the present work using the bioinformatics approach the interactions of miRNA with mRNA of HSF genes *A. thaliana*, *O. sativa*, *T. aestivum*, *Z. mays* were studied. The results of the following research are: a) the miRNA binding sites for the mRNA genes of the HSF family of *A. thaliana*, *O. sativa*, *T. aestivum*, *Z. mays* were established; b) The miRNA binding sites are located in 5'UTR, CDS and 3'UTR mRNA target genes; c) specific miRNAs for different plant species were detected which affect the expression of heat stress transcription factors.

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