

Features of the interaction of miRNAs with genes of the rice MYB family under stress

A.T. Ivashchenko*, A.K. Rakhmetullina, A.U. Pyrkova

Al-Farabi Kazakh National University, Almaty, Kazakhstan

DOI 10.18699/ICG-PlantGen2019-33

© Autors, 2019

* e-mail: a.iavashchenko@gmail.com

Abstract: Data on the involvement of miRNA in the plant response to stress factors are considered. The MYB family of transcription factors plays a great role in the plant development, metabolism and responses to biotic and abiotic stress. The free energy of miRNA binding, the value of free energy of interaction, the position and schemes of potential binding sites were calculated using the MirTarget program. To identify miRNAs whose targets are genes in the MYB family, a search for binding sites of 738 miRNAs in mRNAs of 124 MYB family genes of *Oryza sativa* was performed. 16 genes were identified as targets for 11 miRNAs. The results show that miRNA can regulate the expression of most MYB genes and thus affect productivity and sustainability.

Key words: miRNA; mRNA; genes; transcription factors; rice; stress.

1. Introduction

Rice is one of the main food crops. According to the Food and Agriculture Organization of the United Nations, rice is the staple food for more than half of the world's population. MYB proteins constitute one of the largest transcription factor families in the plant kingdom, members of which are key factors in regulatory networks controlling development, metabolism and responses to biotic and abiotic stresses in the plant genome (Shushi et al., 2015). In agricultural production, abiotic stresses are known as the main causes leading to lower yields (Hoang et al., 2017). The plants have developed complex mechanisms for overcoming various stresses (Jian et al., 2010). Recent evidence suggests that miRNAs are involved in the regulation of gene expression, which affects the post-transcriptional stage of gene expression and relates to biotic and abiotic stress reactions in plants (Bari et al., 2014). Stress in plants causes an increase or decrease in the expression of certain miRNAs or the synthesis of new miRNAs (Varsha et al., 2016). Several stress-regulated miRNAs have been found in plants under various biotic and abiotic stressful conditions, including nutrient deficiency (Fujii et al., 2005), drought (Zhao et al., 2007; Liu et al., 2008; Zhou et al., 2010), low temperatures (Zho et al., 2008), salinity (Sunkar et al., 2008), bacterial infection (Navarro et al., 2006), UV-B radiation (Zhou et al., 2007), and mechanical stress (Lu et al., 2005). These studies suggest that miRNA is regulated in various ways in response to stress. However, limited studies have reported an association of miRNA with the expression of plant MYB genes. In the present work, the task was set to identify, using bioinformatics approaches, miRNAs that can bind to mRNA genes of the MYB family of rice and regulate their expression.

2. Materials and methods

The object of the study was the completely sequenced *O. sativa* genome. The nucleotide sequences of the mRNA genes of the MYB family were obtained from PlantTFDB, a plant transcription factor database (planttfdb.cbi.pku.edu.cn/). The miRNA nucleotide sequences were borrowed from miRBase

(<http://www.mirbase.org/>). The free energy (ΔG) of miRNA binding, the position and schemes of potential binding sites were calculated using the MirTarget program (Ivashchenko et al., 2016). ΔG_m for miRNA was defined as the free energy of miRNA binding with its fully complementary nucleotide sequence. The miRNA binding sites with mRNA were selected with the $\Delta G/\Delta G_m$ ratio being more than 90 %. Unique features of the MirTarget program include the consideration of the nucleotide interaction in miRNA with the mRNA of target genes not only between adenine (A) and uracil (U), guanine (G) and cytosine (C), G-U, but also between A and C through a single hydrogen bond based on the fact that the distance between A and C is equal to the distance between the nucleotides G-C, A-U, G-U (Leontis et al., 2002; Kool et al., 2001).

3. Results and discussion

The study of the binding of 738 miRNA to mRNA of 124 genes of the MYB *O. sativa* family revealed that only 16 genes were targets for 11 miRNA (Table 1).

Four miRNA of the miR159c,d,e,f-3p family had the largest number of binding sites which were associated with mRNA genes (LOC_Os06g40330.1, LOC_Os01g59660.1, LOC_Os05g41166.1, LOC_Os03g38210.1, LOC_Os04g46384.1) of the MYB family with the $\Delta G/\Delta G_m$ value from 90 to 96 %. Therefore, miR159 involved in the response to stress (Varsha et al., 2016) can affect the expression of the rice genes studied. miR2102-5p was binding with mRNA genes (LOC_Os03g26130.1, LOC_Os01g64360.1, LOC_Os01g62410.1, LOC_Os03g25550.1) with the energy from -110 kJ/mole to -113 kJ/mole and the degree of complementarity ($\Delta G/\Delta G_m$) 91 % and 93 %. miR5075-3p had two binding sites in the mRNA of MYB genes. miR171d-5p, miR172d-5p; miR5827-5p had only one target gene (LOC_Os03g27090.1, LOC_Os04g42950.1, LOC_Os06g02250.1, LOC_Os06g46560.1, LOC_Os01g18240.1) with the value of $\Delta G/\Delta G_m$ from 91 to 93 %. The miRNA binding sites in the mRNA genes of the MYB *O. sativa* family were located in the protein coding region.

Table 1

Characteristics of miRNA BSs in the coding region mRNA of MYB transcription factors genes of *O. sativa*

Gene	miRNA	Start	ΔG , kJ/mole	$\Delta G/\Delta G_m$, %
LOC_Os06g40330.1	miR159c-3p	1418	-102	92
LOC_Os01g59660.1	miR159c-3p	1343	-102	92
LOC_Os05g41166.1	miR159c-3p	878	-106	96
LOC_Os03g38210.1	miR159d-3p	938	-102	91
LOC_Os05g41166.1	miR159d-3p	878	-104	92
LOC_Os05g41166.1	miR159e-3p	878	-102	92
LOC_Os06g40330.1	miR159f-3p	1418	-100	90
LOC_Os04g46384.1	miR159f-3p	199	-100	90
LOC_Os01g59660.1	miR159f-3p	1343	-104	94
LOC_Os03g27090.1	miR171d-5p	369	-106	91
LOC_Os04g42950.1	miR172d-5p	995	-96	90
LOC_Os03g26130.1	miR2102-5p	661	-110	91
LOC_Os01g64360.1	miR2102-5p	453	-110	91
LOC_Os01g62410.1	miR2102-5p	441	-110	91
LOC_Os03g25550.1	miR2102-5p	804	-113	93
LOC_Os04g38740.1	miR5075-3p	727	-113	91
LOC_Os08g34960.1	miR5075-3p	553	-117	95
LOC_Os06g02250.1	miR528-5p	523	-106	91
LOC_Os06g46560.1	miR5819-5p	777	-115	93
LOC_Os01g18240.1	miR5827-5p	604	-98	92

Gene, miRNA, site, region of mRNA, characteristics of binding
LOC_Os06g40330.1; miR159c-3p; 1418; CDS; -102; 92; 21 5' -UAGAGCUC CCUUCACUCCA AAU-3' 3' -ACCUCGAGGGAAGUUAGGUUA-5'
LOC_Os05g41166.1; miR159c-3p; 878; CDS; -106; 96; 21 5' -UGGAGCUC CCUUAUCCA AAU-3' 3' -ACCUCGAGGGAAGUUAGGUUA-5'
LOC_Os03g38210.1; miR159d-3p; 938; CDS; -102; 91; 21 5' -CCGAGCUC CCUCCAAGCCA AAU-3' 3' -GCCUCGAGGGAAGUUAGGUUA-5'
LOC_Os05g41166.1; miR159d-3p; 878; CDS; -104; 92; 21 5' -UGGAGCUC CCUUAUCCA AAU-3' 3' -GCCUCGAGGGAAGUUAGGUUA-5'
LOC_Os01g59660.1; miR159f-3p; 1343; CDS; -104; 94; 21 5' -UGGAGCUC CCUUCACUCCA AAG-3' 3' -AUCUCGAGGGAAGUUAGGUUC-5'
LOC_Os04g38740.1; miR5075-3p; 727; CDS; -113; 91; 21 5' -GCGGGCGGCGCGCGGAGGU-3' 3' -CGCCUGCCGCCGCGCCUCUU-5'

Figure 1. Schemes of miRNA interaction with mRNAs of MYB transcription factor genes in *O. sativa*.

Interaction schemes of miRNA with the MYB *O. sativa* mRNA genes (Figure 1), obtained using the miRTarget program, clearly show connections between their complementary nucleotides. Above each scheme is the name of the gene, the name of miRNA, the position of the beginning of the binding site (nt), the binding energy (kJ/mol), the value $\Delta G/\Delta G_m$ (%) and the length of mRNA (nt). The upper and lower nucleotide sequences indicate mRNA and miRNA, respectively.

4. Conclusions

As a result of the research for each miRNA, groups of target genes of MYB transcription factors were 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. The associations found between miRNAs and genes can be used as markers of control of physiological processes in selection and regulation of growth of plant that are highly productive and resistant to abiotic and biotic stresses.

References

Bari A., Sagaidak I., Pinskii I., Orazova S., Ivashchenko A. Binding of miR396 to mRNA of Genes Encoding Growth-Regulating *Transcription Factors* *Plants*. 2014;6(6):807–810. DOI 10.1134/S1021443714050033.

Fujii H., Chiou T.J., Lin S.I., Aung K., Zhu J.K. A miRNA involved in phosphate-starvation response in Arabidopsis. *Curr. Biol.* 2005; 15(22):2038–2043. DOI 10.1016/j.cub.2005.10.016.

Hoang X., Nhi D., Thu N., Thao N., Tran L. Transcription factors and their roles in signal transduction in plants under abiotic stresses. 2017;18(6):483–497. DOI 10.2174/1389202918666170227150057.

Ivashchenko A.T., Pyrkova A.Y., Niyazova R.Y., Alybayeva A., Baskakov K. Prediction of miRNA binding sites in mRNA. *Bioinformatics*. 2016;12(4):237–240.

- Kool E.T. Hydrogen bonding, base stacking, and steric effects in DNA replication. *Annu Rev Biophys Biomol Struct.* 2001;30:1–22. DOI 10.1146/annurev.biophys.30.1.1.
- Leontis N.B., Stombaugh J., Westhof E. The non-Watson-Crick base pairs and their associated isostericity matrices. *Nucleic Acids Res.* 2002;30(16):3497–3531. DOI 10.1093/nar/gkf481.
- Liu H.H., Tian X., Li Y.J., Wu C.A., Zheng C.C. Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. *RNA.* 2008;14(5):836–843. DOI 10.1261/rna.895308.
- Lu S., Sun Y.H., Shi R., Clark C., Li L., Chiang V.L. Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from *Arabidopsis*. *Plant Cell.* 2005;17(8):2186–2203. DOI 10.1105/tpc.105.033456.
- Navarro L., Dunoyer P., Jay F., Arnold B., Dharmasiri N., Estelle M., Voinnet O., Jones J.D. A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Sci.* 2006;312(5772):436–439. DOI 10.1126/science.1126088.
- Shushi S., Amit K., Viswanathan Ch., Dev M.P., Kailash C.B. Transcriptional Regulatory Network Analysis of MYB Transcription Factor Family Genes in Rice. *Front Plant Sci.* 2015;6:1157. DOI 10.3389/fpls.2015.01157.
- Sunkar R., Zhou X., Zheng Y., Zhang W., Zhu J.-K., Identification of novel and candidate miRNAs in rice by high throughput sequencing. *BMC Plant Biol.* 2008;29(8):25. DOI 10.1186/1471-2229-8-25.
- Zhao B., Liang R., Ge L., Li W., Xiao H., Lin H., Ruan K., Jin Y. Identification of drought-induced microRNAs in rice. *Biochem. Biophys. Res. Commun.* 2007;354(2):585–590. DOI 10.1016/j.bbrc.2007.01.022
- Zhou L., Liu Y., Liu Z., Kong D., Duan M., Luo L. Genome-wide identification and analysis of drought-responsive microRNAs in *Oryza sativa*. *J. Exp. Bot.* 2010;61(15):4157–4168. DOI 10.1093/jxb/erq237.
- Zhou X., Wang G., Zhang W. UV-B responsive microRNA genes in *Arabidopsis thaliana*. *Mol. Syst. Biol.* 2007;3:103. DOI 10.1038/msb4100143.
- Zhou X., Wang G., Sutoh K., Zhu J.-K., Zhang W. Identification of cold-inducible microRNAs in plants by transcriptome analysis. *Biochim. Biophys. Acta.* 2008;1779(11):780–788. DOI 10.1016/j.bbagr.2008.04.005.

Conflict of interest. The authors declare no conflict of interest.