Original Article

In Silico Analysis of Osa-miR164 Gene Family in Rice (Oryza Sativa)

Vuong Quang Tien1,a, Nguyen Huy Duong2,b, Nguyen Lam Phuc1, Phan Minh Vu1, Dao Trong Nhan1, Do Thi Phuc1,*

1VNU University of Science, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam
2Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

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Abstract: MicroRNA (miRNA) is a small (~22 nucleotides) non-coding RNA molecule, which functions in post-transcriptional regulation of gene expression. Previous reports have shown that miRNA plays an important role on the resistance ability of plants to adverse conditions. Rice (Oryza sativa) is a major food crop. In this research, we focused on miR164 family in rice. By using bioinformatics approach, we analyzed sequences of all osa-miR164 belonging to rice miR164 family, evaluated the expression profile of osa-miR164 under different stress conditions, predicted cis-regulatory elements on osa-miR164 gene promoters, and simultaneously predicted miR164-targeted genes and their expressions. The results showed the high conserve in mature osa-miR164 sequences but not in the precursor sequences, different expression pattern of osa-miR164 gene members under stress conditions and various cis-regulatory elements present in osa-miR164 gene promoters which may explain for diverse expression pattern of osa-miR164 genes. Some potential target genes of osa-miR164 were identified and their expressions under different stress conditions were analyzed.

Keywords: miR164, microRNA, non-coding RNA, rice, Oryza sativa.

1. Introduction

MicroRNAs are small non-coding RNA molecules containing about 22 nucleotides that function in post-transcriptional regulation of gene expression [1]. miRNAs play key roles in animals and plants, by promoting cleavage or translation inhibition of targeting mRNAs [1]. Lin-4 was the first miRNA detected in Caenorhabditis elegans [2], and in plant, early miRNAs were detected in Arabidopsis thaliana [3]. miRNAs negatively regulate mRNA by guiding the cleavage while being active in RISC complex (RNA-induced silencing complex) and suppressing protein synthesis or degrading targeting mRNAs [4].

MiR164 family in plants consist of miRNAs with conserved sequences which were found in several species and were one of the first cloned miRNAs in Arabidopsis [3]. In Arabidopsis, miR164s mark cleavage site of mRNAs

1a,b These authors contributed equally to this work.
* Corresponding author.
E-mail address: dothiphuc@hus.edu.vn
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corresponding with targeting NAC genes [NAC genes include: no apical meristem (NAM), Arabidopsis transcription activation factor (AtAF) and cup-shaped cotyledon (CUC)] [5]. In Arabidopsis miRNA164 family has 3 members (ath-miR164a/b/c) which target five NAC genes (CUC1/At3g15170, CUC2/At5g53950, NAC1/At1g56010, At5g07680, and At5g61430). Some studies showed that these NAC genes are required for lateral root development, expansion of the boundary domain, regulation of the age-dependent cell death, and formation of vegetative and floral organs [6-8].

In rice, there are 6 gene members in miR164 family, consisting of osa-miR164a/b/c/d/e/f [9]. It is suggested that miR164-targeted NAC (OMTN) genes may act as negative regulators of drought tolerance in rice with overexpression of OMTN2, OMTN3, OMTN4, and OMTN6 caused increased drought sensitivity in transgenic plant [10].

In this study, we aim to investigate the rice miR164 genes and their targets in response to stress conditions by employing the bioinformatic approach to research on expression profile of osa-miR164 genes under different stress treatments, expression regulation of osa-miR164 genes by cis-regulatory elements present in gene promoters, and miR164-targeted genes’ expressions under different stresses.

2. Methodology

2.1. Genomic Analysis

All stem-loop, mature sequences and locations of osa-miR164 family were obtained from the miRBase database (http://www.mirBase.org/) [11]. These sequences were aligned by CLUSTALW [12] using MEGA7 [13].

2.2. Identification of Potential Promoters of Osa-miR164s

For promoter prediction, the upstream sequences pre-microRNAs of each osa-miR164 gene were downloaded from the rice genome database (http://rice.plantbiology.msu.edu/). The 1500 bp sequence of 5’ to the pre-miRNA was retrieved as the promoter region. The TSSs (Transcription Starting Site) were predicted by the YAPP Eukaryotic core promoter predictor (http://www.bioinformatics.org/yapp/cgi-bin/yapp.cgi), a tool to scan for canonical core promoter elements - BREs (B recognition element), TATA boxes, INRs (initiator motif) and DPEs (downstream promoter element), and synergistic combinations of these elements. The obtained promoters were the ones closest to the 5’ of the pre-miR164.

2.3. Analysis of Cis-regulatory Elements of Rice Osa-miR164 Family

Potential promoter regions (the 1500 bp of 5’ region upstream of the pre-miRNAs) were used to predict the cis-regulatory elements and motifs. The SOGO NEW PLACE database (https://sogo.dna.affrc.go.jp/cgi-bin/sogo.cgi?lang=en), a Database of Plant Cis-acting Regulatory DNA Elements, was used to analyze the cis-regulatory elements of the osa-miR164 family.

2.4. Genes Target Prediction and Their Functions

Mature osa-miR164 sequences of Oryza sativa were downloaded from miRBase. The target genes of osa-miR164a/b/c/d/e/f were identified by using psRNATarget (Version 2017) with the default parameters input, except a more stringent cut-off threshold of expectation ≤ 2. The RAP-DB database was used to confirm the information of targeted genes. Uniprot database was used to determine functions of osa-miR164 targeted genes.

2.5. Expression of Osa-miR164 in Abiotic Stress Conditions

The expression profiles of osa-miR164a/b/c/d/e/f were extracted from PmiRExAt@NABI database [14]. The relative expression level of the gene was presented as the log2 fold change between the control conditions and the different treatment or stress conditions.
2.6. Expression of Osa-miR164 Targeted Genes under Abiotic and Biotic Stresses

To show the expression level of targeted genes under abiotic and biotic stresses, online TENOR and RiceXPro databases were employed to get data experiments under stress conditions of osa-miR164 targeted genes.

3. Results and Discussion

3.1. Sequence Comparison of Osa-miR164 Gene Family

Based on miRBase database, there are six genes in rice miR164 family, including osa-miR164a, osa-miR164b, osa-miR164c, osa-miR164d, osa-miR164e, osa-miR164f. Osa-miR164a, osa-miR164b, osa-miR164c, osa-miR164d, osa-miR164e, osa-miR164f locate in Chr7: 28523341-28523496, Chr5: 15896163-15896271, Chr5: 17327440-17327558, Chr2: 33143567-33143660, Chr3: 10542157-10542288 and Chr5: 23343908-23344117, respectively. Mature sequences of osa-miR164a, osa-miR164b, osa-miR164f are 100% identical (Figure 1A). However, osa-miR164c and osa-miR164d have one different nucleotide from osa-miR164a, b, f (14th base of osa-miR164c is U, and 21st base of osa-miR164d is U), and osa-miR164e has 2 different nucleotides (20th base is A and 21st base is G) (Figure 1A).

![Figure 1. A - Alignment of the mature osa-miR164 sequences in rice. Black color indicates identical nucleotides among six osa-miR164s, while fading ones show lower similarity among six osa-miR164s. B - Alignment of the precursor osa-miR164 sequences. Asterisk marks identical nucleotide.](image-url)
Since the sequences of mature osa-miR164 are highly conserve, we further analyze the sequences of osa-miR164 precursors. The results showed that the osa-miR164 precursor sequences were completely different (not include mature sequences). They shared only 1 nucleotide C (5th base upstream mature sequences) and 1 nucleotide G (2nd base upstream mature sequences) (Figure 1B).

3.2. Analysis of Cis-regulatory Elements of Osa-miR164 Gene Promoters

The cis-regulatory elements present in osa-miR164 gene promoters were shown in Figure 2 and Table 1. MYC, MYB and CuRe motifs (involved in response to abscisic acid, regulation of drought and copper, respectively) were common and can be found in all genes with high number, indicating that miR164 gene family is highly regulated by drought, abscisic acid and copper conditions. DRE motif (salt/drought response) was common in osa-miR164c and osa-miR164f genes. They are found at the distal part of 5' regulatory region in osa-miR164c whilst spread across the osa-miR164f gene. SuRe motif (sulfur responsive element) was found in all genes with low amounts, whereas Erd-1 (required for early response to dehydration) was found in all the regulatory regions of the osa-miR164 genes at varying frequencies. LTRE (Low temperature responsive element) was only distributed in osa-miR164b, c, f. 1-box and ASF-1 motif (light regulation element) were widespread in all regulatory regions. Sugar-repressive elements (TATCCAY, A-box, Pyrimidine box, GARE) were distributed differently between six genes, in which they were absent in osa-miR164e. GARE motif was found in osa-miR164d and osa-miR164f whereas A-box was present only in osa-miR164f. Regarding hormone related cis-regulatory elements, ABRE motif (abscisic acid responsive element) was found in 5 genes except osa-miR164b. S-box (important in ABA response) was located only in osa-miR164c. ARF (auxin response) was presented at varying frequencies in all genes and highest in osa-miR164f (Figure 2).

Figure 2. Map of promoter regions of osa-miR164 genes. The corresponding cis-regulatory elements are described in Table 1. Position of transcription start side (TSS) are predicted using YAPP database. The first nucleotide of osa-miR164 precursors is considered as +1.
Table 1. Potential cis-regulatory elements identified in the 5' regulatory sequences of *osa-miR164* gene family. The 1.5 kb of 5' regulatory region was analyzed using PLACE and YAPP databases.

| Cis-regulatory elements | Sequence | Symbol | Function | Reference | MiR164 |
|-------------------------|----------|--------|----------|-----------|--------|
| DRE                     | AGCCGAC  |        | Salt/drought response element | [15] | a 0 b 1 c 5 d 0 e 0 f 12 |
| ABRE                    | (C/A)(ACG (T/C) G(T/C)/G) | | Abscisic acid response element | [16] | a 2 b 0 c 1 d 1 e 2 f 1 |
| MYC                     | CATGTG; CACATG; CANNTG | | Early response to drought and abscisic acid induction | [17] | a 22 b 18 c 14 d 20 e 22 f 17 |
| GARE                    | TAACAA (G/A) | | Gibberellin Responsive element | [16] | a 0 b 0 c 4 d 2 e 0 f 9 |
| CuRE                    | 5'-TTTGC (TG/C/AG)3' | | Copper responsive element and also involved in oxygen response | [18] | a 10 b 4 c 6 d 8 e 10 f 19 |
| SuRE                    | GAGAC | | Sulphur responsive element | [19] | a 1 b 4 c 1 d 2 e 2 f 3 |
| ARF                     | GGTCCAT; TGTCAT | | Auxin response element | [16] | a 1 b 3 c 2 d 1 e 3 f 8 |
| MYB                     | WAACCA; TAACTG; CNGTTR; YAACKG; GGATA; GAACCTG | | Involved in regulation of drought | [20] | a 9 b 13 c 2 d 7 e 6 f 2 |
| Erd 1                   | ACGT | | Early response to dehydration | [15] | a 8 b 0 c 4 d 2 e 8 f 6 |
| Pyrimidine box          | TTTTTC; CCTTTT | | Gibberellin-response cis-element | [21] | a 3 b 1 c 1 d 2 e 0 f 1 |
| TATCCAY motif           | TATCCA | | Involved in sugar repression | [22] | a 1 b 0 c 1 d 1 e 0 f 1 |
| SRE                     | TTATCCA | | Sugar-repressive element | [19] | a 2 b 1 c 0 d 0 e 0 f 0 |
| LTRE                    | ACCGACA; CCGAAA; GTGCGAC | | Low temperature responsive element | [23] | a 0 b 2 c 6 d 0 e 0 f 3 |
| A-box                   | TACGTA | | Sugar repression element | [24] | a 0 b 0 c 0 d 0 e 0 f 2 |
| S-box                   | CACCTC(T) (C/T)A | | Sugar and ABA response | [25] | a 0 b 0 c 1 d 0 e 0 f 0 |
| CMSRE-1                 | TGGACGG | | Carbohydrate Metabolite Signal Response | [26] | a 0 b 0 c 0 d 0 e 2 f 0 |
| ASF-1 motif             | TGGACGG | | Relevant to light regulation. | [27] | a 5 b 1 c 4 d 2 e 1 f 3 |
| I-box                   | GATAAAG | | Light box Element | [28] | a 6 b 4 c 1 d 4 e 2 f 3 |
3.3. Identification of Oa-miR164 Targeted Genes

Nine *osa-miR164* targeted genes were predicted by using psRNATarget, including NAC-domain proteins (*OMTN1*-*OMTN6*), BURP domain protein 4 (*OsBURP 4*), proteophosphoglycan ppg4 and LOC_Os03g47310. (Table 2). Five genes (*OMTN1*-*OMTN5*), BURP domain-containing protein 4 (*OsBURP04*) and proteophosphoglycan ppg4 are regulated by all six *osa-miR164* genes. The other genes (*OMTN 6* and LOC_Os03g47310) are regulated by five of *osa-miR164*, except *osa-miR164c*. Functions of *osa-miR164* targeted genes are identified by using UniProt database, except *OsBURP04*, proteophosphoglycan ppg4 and LOC_Os03g47310 that cannot identify the functions (Table 2).

Table 2. Potential target genes of *osa-miR164* genes with their IDs and functions

| Micro RNA | Target genes ID | Target genes name | Target gene function | Previous research |
|-----------|-----------------|-------------------|---------------------|------------------|
| Osa-miR164a/b/c/d/e/f | LOC_Os02g36880 | NAC1(*OMTN1*) | Controls the rate of transcription of genetic information from DNA to mRNA, by binding to a specific DNA sequence | Expression of OsNAC family genes in drought stress [10] |
| Osa-miR164a/b/d/e/f | LOC_Os04g38720 | *OMTN2* | | |
| Osa-miR164a/b/d/e/f | LOC_Os12g41680 | *OMTN3* | | |
| Osa-miR164a/b/d/e/f | LOC_Os06g46270 | *OMTN4* | | |
| Osa-miR164a/b/d/e/f | LOC_Os06g23650 | *OMTN5* | | |
| Osa-miR164a/b/d/e/f | LOC_Os02g18690 | BURP domain-containing protein 4 | Expressed in stamen | Identify BURP domain-containing genes and response levels to abiotic stresses [29] |
| Osa-miR164a/b/d/e/f | LOC_Os09g37700 | proteophosphoglycan ppg4 | Flowering related gene in wild rice | Expression profiles of the flowering related genes in common wild rice [30] |
| Osa-miR164a/b/d/e/f | LOC_Os08g10080 | *OMTN6* | Controls the rate of transcription of genetic information from DNA to mRNA, by binding to a specific DNA sequence | Expression of OsNAC family genes in drought stress [10] |
| Osa-miR164a/b/d/e/f | LOC_Os03g47310 | transposon protein, putative, CACTA, En/Spm sub-class | Locates in QTLs for Zn, Fe, Al toxicity tolerance in rice [31, 32] |
3.4. Expression Levels of Osa-miR164 Genes in Several Abiotic Stresses

As shown in Figure 3, the expression patterns of osa-miR164 genes were diverse in response to stresses. While osa-miR164c decreased in expression level, other osa-miR164a/b/f/d expression levels showed slightly increase in all stress conditions. The osa-miR164e changed its expression level differently between stress conditions, in which it decreased under drought and salt conditions while slightly increased under cold treatment.

![Figure 3. Heatmap representing expression level of osa-miR164s in several abiotic stresses.](image)

3.5. Expression Levels of Osa-miR164 Targeted Genes in Different Stress Conditions

The expression profiles of eight osa-miR164 targeted genes except LOC_Os03g47310 gene in the shoot and the root under different stress conditions were obtained in TENOR and RiceXPro databases and were visualized as heatmap in Figure 4.

The gene expression in the roots was shown in Figure 4A. In the NAC gene family (OMTN1-OMTN6) OMTN1 up regulated in all stress conditions and showed highest under osmosis, ABA and JA stresses; OMTN5 also up regulated in almost stress conditions, except after 3 h of dry treatment; OMTN4 had slightly changes in expression levels; OMTN3 down regulated under dry, cold, osmotic stress ABA and JA treatments, but up regulated under high salinity and flood conditions; and OMTN6 down regulated in all stress conditions. OsBURP04 up regulated in all stress conditions, whilst PPG 4 down regulated in most of stress conditions, except in high salinity.

![Figure 4. Heatmap representing expression pattern of osa-miR164 targeted genes in adverse environmental conditions in the root samples (A) and the shoot samples (B).](image)
On the other hand, in the shoot (Figure 4B), most of NAC gene family were down regulated under drought, osmosis and ABA stress condition, except OMTN2 was differently up regulated. OsBURP04 up regulated in all stress conditions. PPG 4 down regulated in high salinity, dry, cold treatments, but up regulated in flood, osmotic stress, ABA and JA treatments (Figure 4B).

When comparing the gene expression between the shoot and the root, it shows organ-specific opposite responses suggesting an organ-specific regulators. OMTN 1 upregulated in the root in all stress, but down regulated in the shoot under many stress. In the root, OMTN 2 up regulated in high salinity, down regulated in cold condition, but not in the shoot. OMTN 4 showed up regulation in high salinity, dry, osmotic, JA treatments and down regulation in cold in the root, but in the shoot the expression of OMTN 4 was in opposite direction. OMTN5 up regulated in all stress in the root, but in the shoot its expression decreased in dry, flood, cold, osmotic and ABA treatments. On the contrary, OMTN 6 down regulated in all stress in the root, but up regulated under flood and JA treatment in the shoot. PPG4 gene expression also showed opposite responses between the root and the shoot under high salinity, flood, ABA and JA treatments.

4. Discussion

MicroRNAs are small non-coding RNA molecules that play key roles in growth, development and stress responses of plants. The miRNAs function by selectively regulating the expression of specific target genes. Thus, identification of the potential target genes of miRNAs provides an effective way to investigate the complex mechanisms responsible for stress adaptation.

By using in silico bioinformatics approach, in this study we analyzed miR164 family in rice. The sequences of mature osa-miR164 a/b/c/d/e/f are highly conserve as in agreement with finding of Fang et al., [10], while the sequences of precursors are highly variable. The diversified sequences of miR164 precursors might be of interest for designing primers for quantification of miR164 gene expression levels, especially for osa-miR164 a/b/f which shown completely identical in sequences of the mature ones.

In order to understand how stress stimuli regulate the expression of osa-miR164, we next analyzed the regulatory regions of osa-miR164 genes. The results showed the different combination of the cis-regulatory elements present in the promoter of each osa-miR164, suggesting the diverse expression pattern of osa-miR164 genes in response to different environmental conditions. However, the expression data of osa-miR164s in database is still limited, in which the expression levels of osa-miR164a/b/f cannot be found separately (Figure 3). Some key cis-regulatory elements responsive for abiotic stress (DRE, MYC, MYB, etc.) and hormone related (GARE, ABRE, ARF) were found in osa-miR164 gene promoters, indicating the functional roles of miR164 in stress responses of rice. In the previous study of Zhang et al., (2011) osa-miR164 was shown to up-regulate under radiation stress [33]. The expression of miR164 gene in response to salt stress are different between plant species, in which the up-regulation was observed in Arabidopsis thaliana [34] and Populus trichocarpa [35] but the down-regulation was found in Zea mays [36] and Panicum virgatum [37]. Previous study has reported that auxin signaling by miR164 is important for normal lateral root development [8]. MiR164 expression was consistent with auxin level and its target NAC1 to regulate auxin signaling [38]. To our knowledge, this is the first report on the cis-regulatory elements of osa-miR164 genes.

In the previous research, Fang et al., (2014) had shown that rice miR164s target six NAC genes designated as OMTN1-OMTN6 and other 3 genes named OMT7-OMT9 [10]. In our study, we could find nine osa-miR164 target genes, in which some of them have been reported to be involved in stress responses in rice such as genes encoding NAC-domain proteins (OMTN1-OMTN6), (Table 2 and references herein). The expression
levels of *osa-miR164* targeted genes under different stress conditions were variable between the root samples and the shoot samples and showed organ-specific opposite responses (Figure 4). Thus, it might be meaningful to carry out experiment to investigate the expression levels of *osa-miR164* genes separately in the roots and in the shoots of rice plants.

Overall, rice *miR164* family has six gene members which are regulated by different stress related *cis*-regulatory elements and subsequently they regulate the expression of target genes involved in stress responses of rice plants. The in-depth understanding of the miRNA-guided regulation mechanisms responsible for stress may help unravel the regulatory networks of stress response and may also help in developing new strategies to manipulate rice plants with improved stress tolerance.

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**References**

[1] D. P. Bartel, C. Z. Chen, Micromangers of Gene Expression: the Potentially Widespread Influence of Metazoan microRNAs, Nat. Rev. Genet, Vol. 55, No. 5, 2004, pp. 396-400, https://doi.org/10.1038/nrg1328.

[2] R. C. Lee, R. L. Feinbaum, V. Ambros, The *C. elegans* Heterochronic Gene Lin-4 Encodes Small RNAs with Antisense Complementarity to Lin-14, Cell, Vol. 75, 1993, pp. 843-854, https://doi.org/10.1016/0092-8674(93)90529-Y.

[3] B. J. Reinhart, E. G. Weinstein, M. W. Rhoades, B. Bartel, D. P. Bartel, MicroRNAs in Plants, Genes Dev, Vol. 16, 2002, pp. 1616-1626, https://doi.org/10.1101/GAD.1004402.

[4] J. C. Carrington, V. Ambros, Role of MicroRNAs in Plant and Animal Development, Science, Vol. 80, No. 301, 2003, pp. 336-338, https://doi.org/10.1126/SCIENCE.1085242.

[5] R. Simita, G. Thomas, P. Alexis, B. Thomas, L. Patrick, T. Klaus, Interplay of *miR164*, Cup-shaped Cotyledon Genes and Lateral Suppressor Controls Axillary Meristem Formation in *Arabidopsis thaliana*, Plant J, Vol. 55, 2008, pp. 65-76, https://doi.org/10.1111/J.1365-313X.2008.03483.X.

[6] G. Hui-Shan, X. Qi, F. J. Feng, C. N. Hai, MicroRNA Directs MRNA Cleavage of the Transcription Factor NAC1 to Downregulate Auxin Signals for Arabidopsis Lateral Root Development, Plant Cell, Vol. 17, 2005, pp. 376-1386, https://doi.org/10.1105/TPC.105.030841.

[7] L. Patrick, P. Alexis, M. Halima, T. Jan, MicroRNA Regulation of the CUC Genes is Required for Boundary Size Control in Arabidopsis Meristem Development, Vol. 131, 2004, pp. 4311-4322, https://doi.org/10.1242/DEV.01320.

[8] K. J. Hee, W. H. Ryun, K. Jeong, K. P. Ok, L. I. Chul, C. S. Hee, H. Daeehe, N. H. Gil, Trifurcate Feed-forward Regulation of Age-dependent Cell Death Involving MiR164 in Arabidopsis, Science, Vol. 323, 2009, pp. 1053-1057, https://doi.org/10.1126/SCIENCE.1166386.

[9] R. Sunkar, Z. Zhou, Y. Zheng, W. Zhang, J. K. Zhu, Identification of Novel and Candidate miRNAs in Rice by High Throughput Sequencing, BMC Plant Biol, Vol. 81, No. 8, 2008, pp. 1-17, https://doi.org/10.1186/1471-2229-8-25.

[10] Y. Fang, K. Xie, L. Xiong, Conserved *miR164*-targeted NAC Genes Negatively Regulate Drought Resistance in Rice, J. Exp. Bot, Vol. 65, 2014, pp. 2119, https://doi.org/10.1093/JXB/ERU072.

[11] A. Kozomara, S. Griffiths-Jones, miRBase: Annotating High Confidence microRNAs using Deep Sequencing Data, Nucleic Acids Res, Vol. 42, 2014, pp. D68-D73, https://doi.org/10.1093/NAR/GKT1181.

[12] M. A. Larkin, G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valent, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, D. G. Higgins, Clustal W and Clustal X Version 2.0, Bioinformatics, Vol. 23, 2007, pp. 2947-2948, https://doi.org/10.1093/BIOINFORMATICS/BTM404.

[13] S. Kumar, G. Stecher, K. Tamura, MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets, Mol. Biol. Evol, Vol. 3, 2016, pp. 1870-1874, https://doi.org/10.1093/MOLBEV/MSW054.

[14] A. K. S. Gurjar, A. S. Panwar, R. Gupta, S. S. Mantri, PmiRExAt: Plant miRNA Expression Atlas Database and Web Applications, Database, 2016, https://doi.org/10.1093/DATABASE/BAW060.

[15] K. Nakashima, Y. Ito, K. Y. Shinozaki, Transcriptional Regulatory Networks in Response to
Abiotic Stresses in Arabidopsis and Grasses, Plant Physiol, Vol. 149, 2009, pp. 88-95, https://doi.org/10.1104/PP.10.129791.

[16] B. Mohanty, Promoter Architecture and Transcriptional Regulation of Genes Upregulated in Germination and Coleoptile Elongation of Diverse Rice Genotypes Tolerant to Submergence, Front, Genet, Vol. 0, 2021, pp. 235, https://doi.org/10.3389/FGENE.2021.639654.

[17] M. R. Khan, I. Khan, Z. Ibrar, J. Léon, A. A. Naz, Drought-responsive Genes Expressed Predominantly in Root Tissues are Enriched with Homotypic cis-regulatory Clusters in Promoters of Major Cereal Crops, Crop J, Vol. 5, 2017, pp. 195-206, https://doi.org/10.1016/J.CI.2016.10.001.

[18] S. Labbé, M. M. O. Peña, A. R. Fernandes, D. J. Thiele, A Copper-sensing Transcription Factor Regulates Iron Uptake Genes in Schizosaccharomyces pombe, J. Biol. Chem, Vol. 274, 1999, pp. 36252-36260, https://doi.org/10.1074/JBC.274.51.36252.

[19] A. Maruyama-Nakashita, Y. Nakamura, A. W. Takahashi, E. Inoue, T. Yamaya, H. Takahashi, Identification of a Novel Cis-acting Element Conferring Sulfur Deficiency Response in Arabidopsis Roots, Plant J, Vol. 42, 2005, pp. 305-314, https://doi.org/10.1111/J.1365-313X.2005.02363.X.

[20] J. Li, G. Han, C. Sun, N. Sui, Research Advances of MYB Transcription Factors in Plant Stress Resistance and Breeding, Plant Signal, Behav, Vol. 14, 2019, https://doi.org/10.1080/15592324.2019.1613131.

[21] M. Mena, F. J. Cejudo, I. I. Lamoneda, P. Carbonero, A Role for the DOF Transcription Factor BBPF in the Regulation of Gibberellin-Responsive Genes in Barley Aleurone, Plant Physiol, Vol. 130, 2002, pp. 111-119, https://doi.org/10.1104/PP.005561.

[22] Y. Li, K. K. Lee, S. Walsh, C. Smith, S. Hadingham, K. Sorefan, G. Cawley, M. W. Bevan, Establishing Glucose- and ABA-regulated Transcription Networks in Arabidopsis by Microarray Analysis and Promoter Classification Using a Relevance Vector Machine, Genome Res, Vol. 16, 2006, pp. 414-427, https://doi.org/10.1101/GR.4237406.

[23] S. A. Sheshadri, M. J. Nishanth, B. Simon, Stress-Mediated cis-Element Transcription Factor Interactions Interconnecting Primary and Specialized Metabolism in Planta, Front, Plant Sci, Vol. 0, 2016, pp. 1725, https://doi.org/10.3389/FPLS.2016.01725.

[24] N. Kovalchuk, W. Wu, O. Eini, N. Bazanov, M. Pallotta, N. Shirley, R. Singh, A. Ismagul, S. Elibly, A. Johnson, P. Langridge, S. Lopato, The Scutellar Vascular Bundle-specific Promoter of the Wheat HD-Zip IV Transcription Factor Shows Similar Spatial and Temporal Activity in Transgenic Wheat, barley and Rice, J. Plant Biotechnol, Vol. 10, 2012, pp. 43-53, https://doi.org/10.1111/J.1467-7652.2011.00633.X.

[25] G. J. Acevedo-Hernández, P. León, L. R. H. Estrella, Sugar and ABA Responsiveness of a Minimal RBCS Light-responsive Unit is Mediated by Direct Binding of ABI4, Plant J, Vol. 43, 2005, pp. 506-519, https://doi.org/10.1111/J.1365-313X.2005.02468.X.

[26] A. Morikawa, R. Matsunaga, Y. Tanaka, S. Suzuki, S. Mano, K. Nakamura, Two cis-acting Regulatory Elements are Involved in the Sucrose-inducible Expression of the Sporamin Gene Promoter from Sweet Potato in Transgenic Tobacco, Mol, Genet, Genomics, Vol. 2726, No. 272, 2005, pp. 690-699, https://doi.org/10.1007/S00438-004-1100-Y.

[27] F. Xu, X. H. Huang, L. L. Li, G. Deng, H. Cheng, X. F. Rong, J. B. Li, S. Y. Cheng, Molecular Cloning and Characterization of GbDXS and GbGGPPS Gene Promoters from Ginkgo biloba, Genet, Mol, Res Vol. 12, 2013, pp. 293-301, https://doi.org/10.4238/2013.FEBRUARY.4.3.

[28] A. Rose, I. Meier, U. Wiemand, The Tomato I-box Binding Factor LeMYBI is a Member of a Novel Class of Myb-like Proteins, Plant J, Vol. 20, 1999, pp. 641-652, https://doi.org/10.1046/J.1365-313X.1999.00638.X.

[29] X. Ding, X. Hou, K. Xie, L. Xiong, Genome-wide Identification of BURP Domain-containing Genes in Rice Reveals a Gene Family with Diverse Structures and Responses to Abiotic Stresses, Planta, Vol. 2301, No. 230, 2009, pp. 149-163, https://doi.org/10.1007/S00425-009-0929-Z.

[30] J. Wang, Y. Long, J. Zhang, M. Xue, G. Huang, K. Huang, Q. Yuan, X. Pei, Combined Analysis and miRNA Expression Profiles of the Flowering Related Genes in Common Wild Rice (Oryza rufipogon Griff), Genes Genomics, Vol. 40, No. 8, 2018, pp. 835-845, https://doi.org/10.1007/s13258-018-0688-y.

[31] J. Zhang, K. Chen, Y. Pang, S. A. Naveed, X. Zhao, X. Wang, Y. Wang, M. Dingkuhn, J. Pasaquin, Z. Li, J. Xu, QTL Mapping and Candidate Gene Analysis of Ferrous Iron and Zinc Toxicity Tolerance at Seedling Stage in Rice by Genome-wide Association Study, BMC Genomics, Vol. 27, No. 18, 2017, pp. 828, https://doi.org/10.1186/s12864-017-4221-5.
[33] M. Zhang, S. Liang, X. Hang, Y. Xiang, Z. Cheng, W. Li, J. Shi, L. Huang, Y. Sun, Identification of Heavy-ion Radiation-induced MicroRNAs in Rice, Adv. Sp. Res, Vol. 6, 2011, pp. 1054-1061, https://doi.org/10.1016/J.ASR.2010.10.024.

[34] B. B. Amor, S. Wirth, F. Merchán, P. Laporte, Y. A. Caraño, J. Hirsch, A. Maizel, A. Mallory, A. Lucas, J. M. Deragon, H. Vaucheret, C. Thermes, M. Crespi, Novel Long Non-protein Coding RNAs Involved in Arabidopsis Differentiation and Stress Responses, Genome Res, 19, 2009, pp. 57, https://doi.org/10.1101/GR.080275.108.

[35] B. Li, H. Duan, J. Li, X. W. Deng, W. Yin, X. Xia, Global Identification of miRNAs and Targets in *Populus euphratica* under Salt Stress, Plant Mol, Biol, Vol. 816, No. 81, 2013, pp. 525-539, https://doi.org/10.1007/S11103-013-0010-Y.

[36] D. Ding, L. Zhang, H. Wang, Z. Liu, Z. Zhang, Y. Zheng, Differential Expression of miRNAs in Response to Salt Stress in Maize Roots, Ann, Bot, Vol. 103, 2009, pp. 29, https://doi.org/10.1093/AOB/MCN205.

[37] F. Xie, Q. Wang, R. Sun, B. Zhang, Deep Sequencing Reveals Important Roles of microRNAs in Response to Drought and Salinity Stress in Cotton, J. Exp, Bot, Vol. 66, 2015, pp. 789-804, https://doi.org/10.1093/JXB/ERU437.

[38] Q. Liu, Y. Q. Chen, Insights into the Mechanism of Plant Development: Interactions of miRNAs Pathway with Phytohormone Response, Biochem, Biophys, Res, Commun, Vol. 384, 2009, pp. 1-5, https://doi.org/10.1016/J.BBRC.2009.04.028.