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Molecular Biology

Evaluation of miR398 Differential Expression in Rice under Drought Stress Condition

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ABSTRACT. Drought stress is one of the most important abiotic stresses that deteriorates rice agriculture. One of the best ways to establish drought stress tolerance in plants is miRNA (microRNA) mediated post transcriptional gene regulation. MiRNAs are small non-coding RNAs with 19-24 nt length that are originated from MIRNA genes. MiR398 is one of the important stresses responsive miRNAs. It has been reported that this miRNA could play important role under stress conditions. In this work we studied miR398 differential expression under drought stress in both shoot and root tissues of rice using qRT-PCR. Results indicated that miR398 showed significant up-regulation in shoot tissue whereas no change was detected for this miRNA expression in root tissue under drought stress. In addition, we observed MYB and ERE in the up-stream regions of MIRNA genes that justify miR398 differential expression under drought stress. The results of target prediction indicated that miR398 could regulate Cu-Zn superoxide dismutase. Thus, miR398 could play important role under drought stress condition. © *2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *microRNA,* Oryza sativa*, drought stress, root, shoot, qRT-PCR*

Drought stress is one of the major abiotic stresses that deteriorates rice agriculture. Drought stress resulted in harmful effects on metabolic processes including stomatal closure, nutrient absorption and production of Photosynthetic assimilates that could lead to reduced yield [1, 2]. When plants face stress, several mechanisms in different physiological, biochemical and molecular levels are employed that leads to avoidance or tolerance of plants to drought stress [3]. Transcriptional regulatory mechanisms that could regulate genes expression have been studied [4, 5]. After identification of small RNAs, researchers tend to regulate gene expression using small RNAs like miRNAs (microRNAs) under drought stress [1]. miRNAs are small non-coding RNAs with 19-24 nt length that are originated from MIRNA genes. Primary miRNAs (pri-miRNAs) are transcripted by RNA polymerase II from MIRNA genes in the miRNA

Table1. Designed primers for evaluation of miR398 expression

MiRNA	Members	Reverses primer	Forward primer	Stem-loop RT PCR primer
MiR398	MiR398a,b	5' GTGCAGGGTCCGAG GT 3'	5' GCTCTGTGTTCTCAGGTC AC 3'	5'GTCGTATCCAGTGCAG GGTCCGAGGTATTCGCA CTGGATACGACCAAGGG 3'

biogenesis pathways. Processing of pri-miRNAs to precursor miRNA (pre-miRNA) occurs through Dicer like1 (DCL1) cleavage with HYL1 and SE proteins cooperation. Pre-miRNAs have stem-loop structure that produce mature miRNAs through DCL1 next cleavage processing [6]. miRNAs are involved in different biological and metabolic processes. miRNAs are also involved in developmental programs including auxin signaling, lateral root development, meristematic boundaries formation, organ differentiation, leaf polarity and development, transition from vegetative to reproductive stage [2]. Genes expressions could be regulated in negative and positive ways through miRNAs regulations. Some of miRNAs are negative regulators up-regulated under drought stress that could down-regulate their target genes. On the other hand, some miRNAs are positive regulators. Their expression could be led to their targets of up-regulation under drought stress. Therefore, miRNAs induce adaptation to plants through their positive and negative regulatory systems. In addition, several numbers of miRNAs could regulate genes that encode transcription factors. These features placed miRNAs in the center of gene expression regulation networks [7]. Several studies have been done for identifying miRNA differential expression under drought stress conditions that lead to identification of the drought responsive miRNAs including miR156, miR159, miR167, miR168, miR169, miR171, miR393, miR396 and miR397 [3]. MiR398 is one of the important stresses responsive to miRNA. It is reported that miR398 plays important role in oxidative stress, drought stress, ABA, salt stress, copper and phosphate deficiency and biotic stress [8]. Kantar et al. observed miR398 up-regulation under drought stress using microarray in *triticum dicoccoides* [3]. Likewise,Trindade et al. observed miR398 up-regulation under drought stress using northern blot in *Medicago truncatula* [10]. But conversely, Wang et al. observed miR398 down-regulation under drought stress using deep-sequencing in *Medicago truncatula* [11]. These results indicated the importance of miR398 in regulatory system under drought stress. In this study, we evaluate miR398 expression under normal and drought stress in both shoot and root tissues of rice.

Materials and Methods

Plant materials: IR64 genotype used in this study was derived from the international rice institute. Rice seeds germinated in petri dishes and then were transferred to Yoshida hydroponic culture solution. After two weeks, seedlings were moved to pots containing two parts of sand and one part of clay and were well watered for two weeks. For drought treatment irrigation was stopped for two weeks while for normal treatments irrigation was continued. After two weeks, sampling was conducted and all root tissues were stored at -80.

qRT-PCR: miR398 sequence was downloaded from miRBase (http://mirbase.org). Designed primers are described in table1. Also, 18S rRNA selected as references gene with 5'ATAACTCGACGGATCGCAAG3' and 5'CTTGGATGTGGTAGCCGTTT 3' sequences as forward and reverse primers, respectively. Total RNA was extracted using Trizol reagent. Quality of extracted RNA was evaluated by Nano drop. Later on, *DNase* I treatment was conducted using Invitrogen kit. In addition, cDNA synthesis was carried out using Invitrogen kit. qRT-PCR was conducted in 25µl volume including 12.5 µl of SYBER, 1 µl forward

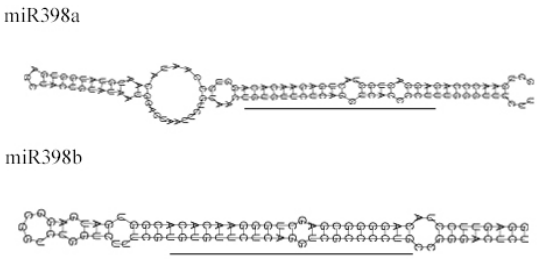

Fig. 1. Stem-loop structure of miR398 family members. Dark lines indicated mature miRNAs production positions.

primer, 1 µl of reverse primer and 3 µl of cDNA. Calculation of miRNA relative frequency and their differential expression was conducted according to Schmittgen *et al.* method of relative quantification [12].

Bioinformatics Analysis: 3000 bp upstream regions of MIRNA genes of *Oryza sativa* were downloaded from plant miRNA database [13] and searched for the existence of important regulatory elements using NSITE-PL version 2.2004(http://www.softberry.com/cgi-bin/programs/promoter/nsite.pl). Furthermore, Mfold software was used for drawing miRNAs stem-loop structures. In order to predict miRNA target genes, we used OSA1 TIGR genome cDNA version 5 (OSA1R5) of psRNATarget (http://plantgrn.noble.org/psRNATarget).

Results and Discussion

According to miRBase data, miR398 is a conserved family with multiple loci in each plant. Osa-miR398 includes two members: miR398a and miR398b. MiR398a and miR398b are located in chromosome number 7 and 10, respectively. Plant miRNA families produce similar mature miRNAs from their stem-loop precursor [14]. We observed both miR398a and miR398b produce 21 nt length mature miRNAs that are different from each other only in 2 nucleotides. Thus, this similarity of both mature miRNAs could be resulted in cooperation of miRNA members in regulation of a target gene (Fig. 1).

In this study, 3000 bp up-stream of MIRNA genes where searched to identify important regulatory elements. More than 50 unique regulatory elements were detected in the up-stream regions of MIR398 genes (Fig. 2). ERE, MYB, GCC-Box where some of important identified regulatory elements in the up-stream regions of MIRNA genes. ERE was observed several times in the up-stream regions of MIR398b. Thus, it is probable that this member of miR398 family is more responsible to ethylene than the other member miR398a. MYB regulatory element was also observed in the up-stream region of MIR398 genes. It was also

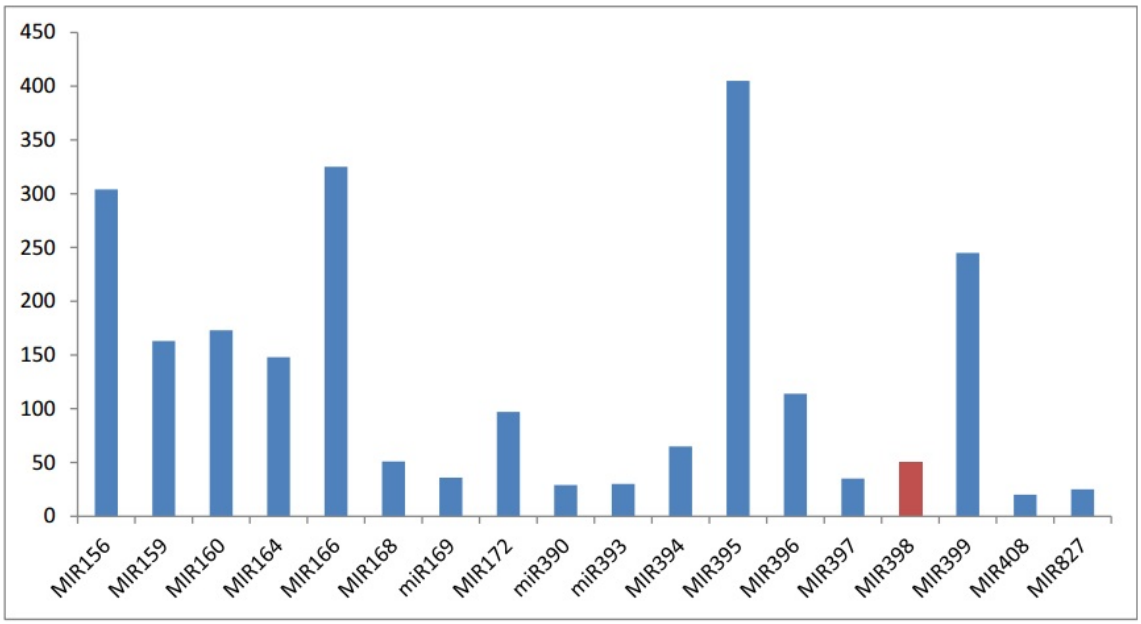

Fig. 2. Frequency of regulatory elements in the up-stream regions of conserved miRNA families. MiR398 is highlighted with red Bar among other families with blue bar.

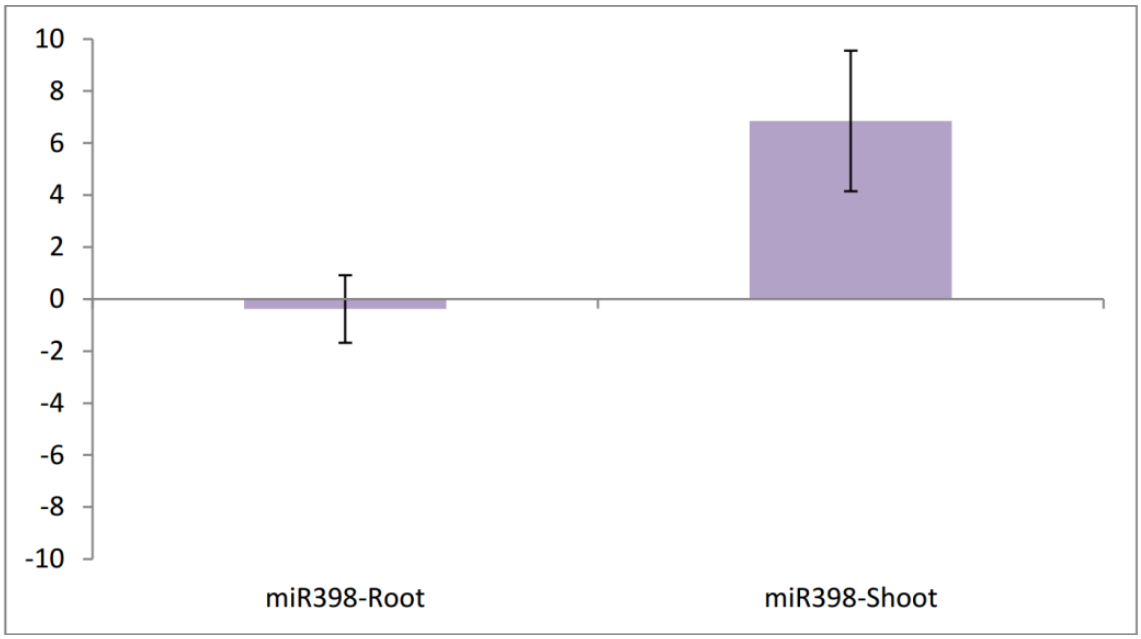

Fig. 3. Differential expression (Fold-change) of miR398 under drought stress in both shoot and root tissues.

observed that MYB regulatory elements in the up-stream regions of some drought responsive genes like rd22. This regulatory element play important roles in regulation of expression of the drought responsive genes [15].

Results of miR398 differential expression indicated that miR398 showed down-regulation in root tissue under drought stress, whereas this miRNA showed up-regulation in shoot tissue under drought stress. We used t-student significant test to evaluate the significance of the expression of this miRNA. Results indicated that differential expression of miR398 is significant in shoot tissue under drought stress in 0.05 % level. We observed that this miRNA changed 6-fold under drought stress in shoot tissue of rice (Fig. 3). Likewise, in several previous reports, miR398 up-regulation has been reported under drought stress for *triticum dicoccoides* and *medicago truncatula* [9, 1] though Wang et al reported miR398 down-regulation in *medicago truncatula* under drought stress [11]. Thus miR398 differential expression in our study in shoot tissue is consistent with most of previous results in other species. However, existence of some in-consistencies could be due to the use of different species or different stress intensity.

Target genes were identified in this study using psRNAtarget online database and results indicated that miR398 members in rice could regulate superoxide dismutase (SOD) especially through cleavage of target genes. Thus, miR398 could play important role in rice shoot tissue than rice root tissue through regulation of SOD.

Kantar et al. and Wang et al. reported that miR398 is involved in Cu-Zn superoxide dismutase regulation, copper homeostasis and response to oxidative stress [9, 11]. In addition, Lu et al., reported ethylene accumulation effects on miR398 expression in rice [13]. On the other hand, we observed existence of several ethylene responsive elements in the up-stream region of MIR398 genes. Therefore, change in ethylene accumulation under drought stress could lead to differential expression of miR398 and then superoxide dismutase. In addition it has been observed that miR398 over-expression resulted in down-regulation of CSD1 and CSD2 and then increasing sensitivity of plant to environmental stresses. But on the other hand, miR398-resistant of CSD2 showed more tolerance to drought and salt stress [16]. In this study, we observed that miR398 showed up-regulation under drought stress. On the other hand, it has been re-

ported that miR398 over-expression leads to CSD down-regulation and increasing sensitivity to drought stress [16]. Thus, it could be concluded that transgenic plants with down-regulated miR398 or miR398-resistant of CSD could increase tolerance to drought in rice.

მოლეკულური ბიოლოგია

ბრინჯის miR398 დიფერენციალური გამოსახულების შეფასება გვალვის ზეგავლენის პირობებში

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(წარმოდგენილია აკადემიის წევრის თ. ბერიძის მიერ)

გვალვა ერთი-ერთი უმნიშვნელოვანესი ფაქტორია, რომელიც დამღუპველად მოქმედებს ბრინჯის მეურნეობაზე. მცენარის გვალვისადმი მედეგობის დასადგენად ერთ-ერთი საუკეთესო გზა გენების პოსტტრანსკიფციული რეგულაციაა miRNA-ს (microRNA) საშუალებით. miRNA წარმოადგენს 19-24ნტ სიგრძის არაკოდირებადი ნუკლეინის მჟავას მცირე მოლეკულებს, რომლებიც MMIRNA-ს გენებისგან წარმოიქმნება. ერთ-ერთ მნიშვნელოვან miRNA-ს წარმოადგენს miR398, რომელსაც შეუძლია დიდი როლი შეასრულოს სტრესის პირობებში. ნაშრომში შესწავლილია miR398-ს დიფერენციალური ფორმულა ბრინჯის მცენარისა და ფესვის ქსოვილებისათვის qRT-PCR-ს გამოყენებით გვალვის ზეგავლენის პირობებში. შედეგებმა აჩვენა, რომ გვალვის პირობებში miR398 მცენარის ქსოვილში მნიშვნელოვან ზერეგულაციას ახდენს, ხოლო ფესვის ქსოვილში ფორმულის არავითარი ცვლილება არ შეინიშნებოდა. ამასთან ერთად MIRNA-ს გენების აღმავალი მიმართულებით MYB და ERE დაფიქსირდა, რაც მოწმობს, რომ გვალვის პირობებში miR398-ს დიფერენციალური ფორმულა გამართლებულია. გარდა ამისა, შედეგებმა გვიჩვენა, რომ miR398-ს შეუძლია დაარეგულიროს Cu-Zn სუპეროქსიდის დისმუტაზა. ამგვარად, გვალვის ზეგავლენის პირობებში miR398-ს მნიშვნელოვანი როლის შესრულება შეუძლია.

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