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Role of Rice miRNAs in Pathogen Response

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Abstract

Micro RNAs (miRNAs) have emerged as the master regulator of many biological functions in both plant and animal systems. In this post-genomic era, studies on miRNA regulation in plants are not limited to *Arabidopsis thaliana*, rather it is shifting towards rice (*Oryza sativa*), one of the potent staple foods for world's inhabitants. Till date, many rice miRNAs have been identified with cloning methods and computational approaches. Huge numbers of potential targets have been identified and some of those have been validated experimentally. Increasing evidence supports that plant miRNAs contribute in immune responses to pathogens. Under different developmental stages and stress conditions, significant changes in the expression profile of miRNAs suggest that they can regulate the production of mRNA targets by cleaving or suppressing. Here we report recent advancement in miRNA mediated regulation in rice and its future direction towards bioengineering.

Keywords: *miRNA; Stress; Pathogens; Bioengineering; Rice*

Introduction

More than half of the world's population depends on rice as a primary source of food and it is also an important model crop. Significant molecular and genetic regulations have been noticed and applied to improve the quantity and quality of rice plant. One of the major factors limiting rice production is the occurrence of diseases caused by various fungal, bacterial and viral pathogens. Rice blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctonia solani*) are the two most devastating fungal diseases of rice. Bakanae ("foolish seedling" in Japanese) caused by the fungus *Fusarium* spp *(Gibberella fujikuroi* species complex) is also common rice disease. Rice yields can be also severely compromised by the bacterial pathogens *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv *oryzicola* (bacterial blight and bacterial leaf streak, respectively). More recently, bacterial foot rot (*Dickeya zeae*, previously known as *Erwinia chrysanthemi* pv. *zeae*) and bacterial panicle blight of rice (*Burkholderia glumae*) have emerged as important pathogens affecting global rice production [1]. Modern techniques are routinely used to identify the key regulatory molecules. Several omics studies for rice have grown significantly in recent years. Though the production of rice under various stresses is not sufficient. We have to dissect the regulatory networks vividly to learn the basic principles behind growth and development of rice plant, how these interact with environment and how these resist the harmful pathogens.

Rice is capable of activating basal resistance against rice blast caused by the fungal pathogen, *Magnaporthe oryzae* by perturbing *OsDCL1*-dependent miRNA biogenesis pathway [2]. Post-transcriptional gene silencing (PTGS) is one of the major regulatory mechanism to maintain the cellular homeostasis [3]. Recent studies have shown that miRNAs have immense role in rice development, abiotic and biotic stress responses etc. The study of miRNAs in rice has progressed from computational prediction to experimental identification and functional characterization. A number of algorithms and tools are available to predict the miRNAs and their targets [4] but the function of individual miRNAs are largely unknown. Studies on genome-wide expression of miRNAs as well as their targets are required to make the present scenario more clear [5]. To reduce the number of false positive miRNA targets, a computational model may be helpful which will depend on experimentally validated data like microarray and next-generation sequencing techniques.

Results

miRNA biogenesis, processing and export

Plant miRNAs are primarily found in genomic regions not associated with protein-coding genes and RNA polymerase II is probably responsible for transcribing most plant miRNAs [6,7]. A central step in miRNA maturation is excising the mature miRNA from the pri-miRNA by RnaseIII-type endonucleases. The processing of pri-miRNAs to pre-miRNAs by DCL1 is assisted by two other proteins, HYPONASTY LEAVES1 (HYL1) and SERRATE (SE) [8,9]. DCL1, HYL1, and SE interact with each other and are co-localized in the nuclear bodies, termed Dicing bodies or D-bodies in which some pri-miRNAs have been found, suggesting that D-bodies serve as a center for active miRNA processing. After the miRNA/miRNA* duplexes are released from the pre-miRNAs by the DCL1 activities, the duplexes are methylated at the 2' OH of the 3' -ends by HUA ENHANCER1 (HEN1), a small RNA methyltransferase. The methylation of the miRNA/miRNA* duplex has been shown to be required to protect miRNAs against the 3' -end uridylation activity and subsequent degradation [10]. The miRNA of the duplexes is selectively incorporated into the RISC, and the miRNA* appears to be removed and subsequently degraded [11]. HASTY, the *Arabidopsis* homolog of exportin 5, is thought to be involved in miRNA nuclear export [12,13]. However, it is unclear whether the HASTY proteins export the miRNA:miRNA* duplexes or the miRNA-RISC complexes in plants.

Mechanism of miRNA function

Forming the miRNA-RISC assembly, miRNAs can direct the RISC to regulate gene expression by two post-transcriptional mechanisms: mRNA cleavage or translational repression [14,15]. The degree of miRNA - mRNA complementarity is a key determinant of the mechanism used. A general rule is that, while perfect or near-perfect complementarity induces mRNA cleavage, central mismatches trigger translational repression [16]. A novel mechanism of gene regulation in rice where miRNAs can target intron region has been reported [17]. It has been demonstrated that miRNAs can cause epigenetic modifications under normal conditions as well as under a diverse array of biotic and abiotic stresses [18,19]. DCL3-dependent 24 nt long miRNAs (l-miRNAs) and hypomethylation of predicted l-miRNA targets Os06g38480, Os03g02010, Os05g01790, Os07g41090 and Os02g05890 in rice DCL3 mutants have been reported [20]. Moreover, the DNA methylation analysis showed that l-miRNAs can also direct DNA methylation at their own miRNA locus in rice.

Regulatory roles

Based on the conservation in other plants rice miRNAs can be grouped into four categories [21] - (i) conserved in at least one non-monocotyledonous plants, (ii) conserved in at least one monocotyledonous plants, (iii) rice specific and (iv) described as non-authentic as they have not been observed in high-throughput RNA sequencing in any plant. These miRNAs have found to be involved a number of regulatory processes.

Stress

Plants exposed to stress use multiple gene regulatory mechanisms, including post-transcriptional regulation of gene expression, to restore and re-establish cellular homeostasis [22]. 74 candidate miRNAs from rice seedlings treated with cold, dehydration, salinity, and abscisic acid (ABA) as well as wild-type seedlings have been reported [23]. Among them OsmiR171b-f has been found ABA, cold and salinity induced library and OsmiR172 a,b,d has been found in cold and salinity induced library. OsmiR1861k was predicted to be inducible by *Xanthomonas oryzae* pv. oryzae (Xoo) in resistant rice leaves by using a computational approach based on transcription data [24].

Abiotic stress

Drought [25], cold [26,27], salinity [28,29], aluminium [30], heavy metal [31], cadmium [32], H_2O_2 [33], arsenite [34], low and high dose rate γ-ray [35] triggers significant changes in expression of miRNAs in rice. Novel and abiotic stress-regulated miRNAs in the inflorescences of rice by high-throughput sequencing have been identified [36]. Trans-acting-small interfering RNAs (Ta-siRNAs) are plant specific molecules generally involved in development and are also stress responsive. miRNA expression profiling reveals that miRNAs which are involved in the progression of plant growth and development are differentially expressed during abiotic stress responses.

Biotic stress

Rice plant frequently remains under threat of different fungus and viruses. Role of plant miRNAs in defence against viruses has been reported [37-39]. miRNAs in the blast fungus *Magnaporthe oryzae* infected rice has been reported [40]. For example, osa-miR7695 negatively regulates an alternatively spliced transcript of OsNramp6 (Natural resistance-associated macrophage protein 6). Multiple miRNAs involved in immunity against *Magnaporthe oryzae* have been reported [41]. RSV (Rice stripe virus) infection enhanced the accumulation of some rice miRNA*s, but not their corresponding miRNAs, as well as accumulation of phased siRNAs from a particular precursor [42]. Furthermore, Rice stripe virus (RSV) infection also induced the expression of novel miRNAs in a phased pattern from several conserved miRNA precursors. 7 novel miRNAs in Deep Sequencing-Based small RNA libraries of RSV-infected rice have been identified [43]. Further they showed that 5 of the 7 new miRNAs were up-expressed and other 2 new miRNAs were down-expressed in RSV-infected rice. Rice miRNA targets against *Tungro* virus were also computationally predicted [44]. Role of miRNAs in five classic plant hormone (auxins, gibberellins (GAs), cytokinins, abscisic acid (ABA) and ethylene) stresses were checked and 11 miRNAs were found to be deregulated by one or more phytohormone treatments [45]. miRNA and target gene pairs implicated in hormone signaling and cross-talk among hormone pathways have great potential in regulating rice immunity [46]. Also existence of a novel regulatory network that integrates miRNA and Conserved-Peptide upstream Open Reading Frame (CPuORF) functions in plants are known. This will help in understanding the underlying regulatory mechanisms of miRNAs in rice immunity. A viral protein hijacks OsDRB1, a key component of the processing complex, for miRNA biogenesis and enhances viral infection and pathogenesis in rice [47]. Microarray-based and next generation sequencing-based transcriptomic approaches have been used to study rice-RSV interactions [48]. Rice long small interfering RNAs (lsiRNAs) are differentially expressed upon infection of *Rhizoctonia solani*, the causal agent of the rice sheath blight disease. Bioinformatic analysis and experimental validation indicate that some rice lsiRNAs can target defense-related genes [49]. miRNAs are involved in rice immunity against *M. oryzae* and that overexpression of *miR160a* or *miR398b* can enhance rice resistance to the disease [41]. Induction of miR319 by *Rice ragged stunt virus* (RRSV) infection in rice suppresses Jasmonic acid (JA) mediated defence to facilitate virus infection and symptom development [50]. A large number of miRNAs and genes showed differential expression in a major disease resistance gene *Xa3/Xa26* mediated resistance [51]. The transfer of low amounts of siRNA, probably occurring passively through the symplastic pathway from the agro-infected area, seemed sufficient to trigger degradation of target transcripts in the adjacent tissues. Two miRNAs that were induced in response to both virus infection and salt-treatment indicate the possibility of similar signaling process due to different types of stress [52]. Thus they concluded that a broad range stress management might be possible by manipulating the levels of only few miRNA.

GM rice and miRNAs

To develop superior crop cultivars with enhanced biotic and abiotic stress tolerance and increased biomass yields; thus increasing the crop yields and quality, miRNA-based genetic modification technology can be one of the promising solution of food security [53]. Rice OsSPL14 can control the grain size, shape, quality [54], panicle branching and higher grain productivity [55]. OsmiR156 can define ideal plant architecture in rice regulating OsSPL [56]. Overexpression of miR319 can enhance cold tolerance [27] and overexpression of OsmiR397 improves rice yield by increasing grain size and promoting panicle branching [57]. Manavalan., *et al.* showed that RNAi-mediated disruption of squalene synthase improves drought tolerance and yield in rice [58]. Warthmann., *et al*. reported that amiRNAs (artificial miRNAs) can efficiently trigger gene silencing in a monocot crop [59]. The targeted genes are specifically down-regulated by amiRNA-guided cleavage of the transcripts, resulting in the expected mutant phenotypes. Down-regulation of starch branching enzyme IIb by the amiRNA technique produced a more extreme starch phenotype in rice [60]. Artificial microRNAs (amiRNAs) mediated gene silencing represents one of such techniques which can utilize as a potential tool in functional genomics. Similar to microRNAs, amiRNAs are single-stranded, approximately 21 nt long, and designed by replacing the mature miRNA sequences of duplex within pre-miRNAs. Artificial microRNAs (amiRNAs) mediated gene silencing represents one of such techniques which can utilize as a potential tool in functional genomics. Similar to microRNAs, amiRNAs are single-stranded, approximately 21 nt long, and designed by replacing the mature miRNA sequences of duplex within pre-miRNAs [61]. Differentially expressed short RNAs (sRNA)s include known microRNAs (miRNAs), unreported miRNAs, and small interfering RNAs. The candidate genes, with expression that was negatively correlated with the expression of sRNAs, were identified, indicating that these genes may be regulated by sRNAs in disease resistance in rice.

Challenges and future perspectives

The above indicates that the study of miRNA biogenesis, regulation and target genes has become a major research topic in case of developmental phases of rice as well as under different stress conditions. In view of the important roles of miRNA in the regulation of gene expression and hence tissue functions and phenotypes, investigations of miRNA offer many opportunities for scientists with bioengineering expertise. One bioengineering application is the use of concepts and techniques for gene targeting to achieve the inhibition of miRNAs *in vitro* and *in vivo*. For gene function identification, sometimes the insert mutant strategy is not effective due to gene functional redundancy. Fortunately, the redundant gene family can be repressed post-transcriptionally in a transgenic plant overexpressing a related miRNA because one miRNA can target multiple mRNAs which are often members of the redundant gene family. By this unique way, we cannot only further confirm the functions of known genes but also probe the functions of those predicted miRNA target genes with unknown functions. Finally, a better understanding on the miRNA regulation mechanisms in plants will make it possible to design artificial miRNAs that may be used as efficient tools for controlling gene expression at will.

A given Transcription factor (TF) may regulate a cluster of miRNAs, which may in turn modulate other TFs as their target genes, thus forming genetic circuits. TF regulation of miRNAs has been studied mainly in cancer cells. In case of rice, the hierarchical relationships of the TF regulation of miRNA and the miRNA regulation of TF are largely unknown. For bio-engineers with expertise in computer-assisted design and analysis, elucidation of transcriptional regulation of miRNA can be a fruitful area of research. In silico analysis of the miRNA promoter regions will be able to facilitate this identification.

Conclusion

Based on the current results obtained from plant degradome sequencing, it has been proposed that there might be a novel self-regulation mechanism of the miRNA genes. The mature miRNAs and the miRNA*s both have their recognition sites on their precursor sequences, i.e. the regions encoding the miRNA*s and the miRNAs, respectively. Thus, the miRNA precursors including pri-miRNAs and pre-miRNAs can be recognized by the corresponding miRNAs or miRNA*s as the *in vivo* targets for degradation. From this point of view, the self-regulation is a mechanism employed by miRNA genes as a buffering system for their expression modulation. This mechanism and the resulting cleavage products can be partially reflected by degradome sequencing data.

An RNA silencing suppressor NS3 has a high affinity for miRNA/miRNA* duplexes, indicating that its activity might also interfere with miRNA-regulated gene expression in both insects and plants [62]. A flagellin derived peptide induces a plant miRNA that negatively regulates mRNAs for the F-box auxin receptors [63]. A debatable matter came out when Zhang., *et al*. reported that rice miRNA 168a specifically targets mammalian LDLRAP1 [64]. They confirmed the evidence of cross-kingdom regulation by miRNA. However, any detectable oral bioavailability of plant miRNAs after feeding in mice was found [65]. Currently, it seems to be a challenging work to establish comprehensive networks with relatively high reliability, since much more experimental data are required. Fortunately, the current research with plant miRNA are encouraging and have greatly advanced in miRNA-implicated gene regulation mechanisms. It is expected that the comparison of miRNA microarray expression profiles with existing mRNA expression data will reveal more regulation relations between miRNAs and their potential targets. For the computational scientists, the preferential issues to be addressed are to collect and analyze the available data, make predictions, validate the hypotheses based on literatures or collaborations with biologists in wet labs and finally establish the network. Several foreseeable difficulties need to be overcome. For example, the accurately defined miRNA promoters could be obtained only after the clarification of the sequence ranges of the pri-miRNAs. Moreover, co-operative bindings of miRNAs may facilitate distinctive and fine-tuned target gene expression patterns [66] and their co-functional activities may help to detect the biological processes [67]. It seems to be still far from achieving comprehensive miRNA-mediated regulatory networks in plants but once established, they will be attractive and valuable for the researchers [68-70].

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Competing Interests

The authors declare that there is no competing interests.

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