Cutting-edge research on plant miRNAs

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The discovery of widespread microRNAs (miRNAs) in animals encouraged the scientists to explore miRNAs and its corresponding roles in plants, which lead to the discovery of incredible physiological functions of these novel small entities in plant systems biology. This discovery made the study on functional analysis of plant miRNA as one of the hottest research topics, globally. The year 2012 was a remarkable year of fascinatign miRNA research, where numerous reports deciphering the plant miRNAs right from its origin and evolution to its diverse functions were published (Box 1). To-date the popular miRNA repository, miRBase, has about 5159 plant entries in its latest release 19 (Griffiths-Jones, pers. comm.).

Identification and characterization of stress-related miRNAs in several plant species were reported more frequently. Gébelin et al.1 identified abiotic stress-related miRNAs in Hevea brasiliensis using deep sequencing and in silico analysis. The information from plant microRNA database (PMRD) and Hevea expressed sequence tag (EST) sequences were retrieved and combined with the LeARN pipeline to identify 10 putatively novel miRNA families and 48 conserved miRNA families. The functional duality of both the families was indicated by the presence of two miRNA class sizes, viz. 24 nt and 23–27 nt. Prediction scanning of both the families was indicated by the presence of two miRNA class sizes, viz. 24 nt and 23–27 nt. Prediction scanning of both the families was indicated by the presence of two miRNA class sizes, viz. 24 nt and 23–27 nt. Prediction scanning of both the families was indicated by the presence of two miRNA class sizes, viz. 24 nt and 23–27 nt. 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MicroRNAs are of 21–22 nt size, specifically involved in regulation of gene expression through RNA interference phenomena. It forms an effector complex and degrades complementary mRNA, thus blocking the mRNA from being translated. miRNAs are denoted as ‘MiR’ followed by the respective number.

Based on the well-established miRNA biogenesis and mode-of-action pathway, transcription of miRNA genes in the nucleus generates pri-miRNA, which is then processed by the Dicer activity of the DCL1/HYL1 (DICER-LIKE1/HYPONASTIC LEAVES1) complex into 21 nt miRNA/miRNA* duplex. The miRNA duplex is further methylated (Me) by the methylase activity of HEN1 (HUA ENHANCER1). Methylated miRNA duplex was exported from the nucleus to cytoplasm assisted by HST (HASTY). In cytoplasm, the mature miRNA is loaded into the AGO1 (ARGONAUTE1) protein, while the miRNA* strand is degraded. The AGO1 protein induces cleavage of target mRNA strand through the ‘slicer’ activity of AGO1.

were found to be high efficiently formed when the miR173 target site was positioned immediately next to the stop codon. Introducing premature stop codons caused a remarkable reduction of tasiRNAs and over-accumulation of 3′ cleavage products, suggesting positive effects of translation on processing the 3′ cleavage products into tasiRNAs. Mutating the end of miR173 target site showed the essentiality of perfect complementarity between the 3′ end of miR173 and the 5′ end of the target sequence, whereas mutation at 5′ end showed insignificant effect. It provided new insights into tasiRNA biogenesis and a strategy for improving the efficiency of RNA interference (RNAi) using tasiRNAs. Manavella et al. demonstrated that the miRNA:miRNA* strand (though 22-nt length are sufficient, it is not essential for this pathway), which is not part of active RISC, and its asymmetrically positioned bulged bases in the duplex can influence miRNA triggered transitivity.

The possibility of utilizing miRNAs for the systematic silencing of plant genes was revealed by Felippes et al. and the phenomenon is termed miRNA-induced gene silencing (MIGS). It was demonstrated in A. thaliana and N. benthamiana, exploiting the ability of non-conserved miR173 to trigger tasiRNA production from its primary target, which is provided on the MIGS vector. The
tasiRNAs can then target an endogenous mRNA and silence them. To facilitate its application, they have also developed a set of suitable vectors that can be used in different plant species. In contrast to other gene silencing strategies such as virus-induced gene silencing (VIGS), hairpin RNA interference (hpRNAi) and artificial miRNA (amiRNAs), MIGS has the benefit of generating vectors easily through a single PCR step\(^9\). Hence MIGS can be effectively utilized in silencing a single gene or multiple unrelated genes for functional genomics studies.

In vitro experimentations on global expression analysis of miRNA involve complementary DNA (cDNA) synthesis and microarray or quantitative real-time PCR (qRT-PCR) assisted with Northern blotting. As reliable quantification of mature miRNA levels is crucial to understand their function, the cDNA synthesis is a highly decisive step in this process. The cDNA synthesis is done either by polyadenylation or hairpin priming (stem-loop RT). Long-term controversies exist in the reliability of both the methods and recently Adhikari et al.\(^{10}\) made a comprehensive study on this aspect and reported that the hairpin priming is better suited than poly-A tailing to generate cDNA for qPCR because the latter might lead to erroneous abundance measurements, possibly depending on the methylation status of mature miRNAs\(^{10}\).

In an interesting study, Zhang et al.\(^{11}\) showed that the ability of plant small RNAs ingested through food can directly affect gene expression in animals upon getting migrated through the plasma and released to tissues. They discovered the presence of an exogenous plant miRNA, MiR168a which is abundant in rice is also present in the sera and tissues of human and mice subjects. Functional studies in vitro and in vivo revealed that MiR168a may attach to the animal low-density lipoprotein receptor adaptor protein 1 ((LDLRAP1) mRNA), hinders its expression in liver and subsequently reduce low-density lipoprotein (LDL) removal from plasma\(^{11}\). However, this report on cross-kingdom regulation by miRNA has been partially disproved by Zhang et al.\(^{12}\). Through computational and experimental analyses, they revealed the occurrence of plant miRNAs in animal sRNA datasets and significantly the MiR168 was exceedingly over-represented. To study the possibility of plant-derived miRNA accumulation and movement in insects, feeding studies for three insects including corn rootworm were conducted. Their analyses ultimately suggested that the observed plant miRNAs in animal sRNA databases can originate in the process of sequencing and the accumulation of plant miRNAs via dietary exposure is not widespread among animals\(^{12}\).