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MicroRNA: A powerful tool for post-transcriptional gene silencing in plants

Mohammed A. M. Aly[*1] and Arjun Sham[2]

[1]Department of Genetics, Faculty of Agriculture, Cairo University, Giza, Egypt
[2]Department of Arid land Agriculture, Faculty of Food and Agriculture United Arab Emirates University, P.O. Box 17555, Al Ain, UAE

***Corresponding author: mohammedaly1@hotmail.com**

Abstract

microRNAs (miRNAs) are extensive classes of ~22-nucleotide non-coding double stranded RNAs that regulate gene expression in metazoans. Plant miRNAs were identified by genetic screening, computational approaches, and expressed sequence tag analysis. Post-transcriptional gene silencing (PTGS) in plants is an RNA-degradation mechanism that requires putative RNA-dependent RNA polymerases and RNA helicases. The sequence-specific degradation of RNA is important in the regulation of protein synthesis and in maintaining signaling traffic. Progress in genomic research of different plant species may open the door for discovering novel miRNAs. The rapid advance in our understanding of miRNAs silencing mechanism will greatly contribute to the study of plant functional genomics. Also, a comparative analysis of these genes, including the gene structure, phylogenetic relationship, conserved protein motifs, gene duplications, chromosomal locations and expression pattern among these plants may shed light on plant development pathways and responses to stress. This review summarizes the current progress in understanding the biogenesis, computational methods involved in miRNAs identification, functions of plant miRNAs genes along with shedding light on the bioinformatics challenges that lie ahead. It also gives an account of plant miRNAs and their versatile roles in plant development. The influence of the advances in genome sequencing of stress tolerant plants such as date palm and jojoba will be briefly addressed in relation to miRNA.

Keywords: Abiotic stress, Biogenesis, Biotic stress, Development, Evolution, Genetic screening, Gene silencing, MicroRNA, Morphogenesis.
Abbreviations: miRN A (MicroRNA), PTGS (Post transcription gene silencing), sRNA (Small RNA), *NAC* (Nitrogen Assimilation Control protein).

Introduction

The discovery of RNA silencing phenomena in plants and animals in the early 1990s (Lee et al., 1993) has dramatically changed our view of RNA and its role in gene expression. The impact of small RNAs (sRNA) in gene silencing has furthered the understanding of gene regulation. One of the first breakthroughs in the study of RNA interference was the transformation of petunia with a sense chalcone synthase transgene which suppressed the expression of both the transgene and the endogenous gene (Napoli et al., 1990; Van der Krol et al., 1990), a process initially referred to as co-suppression. In plants, RNA silencing, as an efficient part of gene silencing, not only serves as an essential component of the defense system being targeted against transposable elements and viral infection, but also plays important roles in the regulation of endogenous gene expression (Voinnet, 2002; Cerutti, 2003). RNA mediated gene silencing is a complex regulatory mechanism that is now known to be involved in such diverse processes as development, pathogen control, genome maintenance and response to environmental changes. Post-transcriptional gene silencing (PTGS) is the accumulation of 21–25 nucleotide small-interfering RNAs, sequence-specific degradation of target mRNAs, and methylation of target gene sequences. Studies on PTGS suggest that this mechanism is highly conserved as several groups of homologous genes are required for silencing in plants, animals, and fungi. Micro RNAs (miRNAs) were first discovered by Lee et al. (1993) as a regulator of larval development in the nematode *Caenorhabditis elegans.* miRNAs are an extensive class of ~22-nucleotide non-coding RNAs thought to regulate gene expression in metazoans (Chicas and Macino, 2001). They are also present in plants, indicating that this class of non-coding (partially double-stranded stem-loop) RNA structures arose early in eukaryotic evolution. Approximately, 3072 plant miRNAs are entered in miRBase 16 releases, which indicate that new miRNAs are being discovered in different plant species. The maximum number of plant miRNAs has been discovered in rice (*Oryza sativa*), where miRNAs have differential expression patterns in development (Reinhart et al., 2002). The plant miRNAs loci potentially encode stem–loop precursors. Numerous plants suffer and/or adapt to biotic and abiotic stresses. Date palm (*Phoenix dactylifera* L.) is one of the most important plants in the arid and semi-arid land as it tolerates extreme adverse environmental conditions, including drought, high temperature and salinity, mostly at the same time. The same applies to jojoba (*Simmondsia chinensis*), another desert plant native to Northern America. Examining miRNAs in date palm and jojoba may facilitate further understanding of miRNAs roles in stress biology. This article gives a brief account of the miRNAs evolution and its applications in plant

development, response to biotic and abiotic stresses and improvement. Date palm (*Phoenix dactylifera* L.) and jojoba (*Simmondsia chinensis*) will be addressed as they tolerate major biotic and abiotic stresses.

Evolution and organization of plant miRNAs

miRNA gene families evolved from the process of gene duplication and diversification that also led to the evolution of protein-coding gene families (Maher et al., 2006). Some miRNAs families, such as miR156, miR160, miR319 and miR390 are conserved from mosses to flowering plants (Arazi et al., 2005; Axtell et al., 2006). Since the complete genome of a non flowering land plant is not currently available, it is not possible to determine how many miRNAs families are conserved among land plants through homology searches (Chen, 2008). Some miRNAs families evolved after mosses and flowering plants diverged before the divergence of monocots and dicots. Most miRNAs have shown to be conserved among related species and homologs were even found among distantly related species. The miRNAs derived from the same gene family are often highly similar. All of the species subjected to high-throughput small RNA (sRNA) sequencing have been found to possess non-conserved miRNAs, which indicates that miRNAs arise continually. Clade-specific miRNAs target a number of functional sites in a genome and species-specific miRNAs are present in a particular species. Clade-specific and species-specific miRNAs target mRNAs having a wider range of functions when compared to the target of conserved miRNAs. For example, the majority of newly discovered non-conserved miRNAs in Arabidopsis (*Arabidopsis thaliana*), target genes involved in metabolism, signal transduction, protein modification and RNA/carbohydrate binding rather than targeting transcription factors (Chen, 2008). The similarity at both the coding and non-coding regions of these miRNAs genes indicates that the expansion of plant miRNAs gene families has a recent origin and may be still ongoing. However, it has also been found that a large set of miRNAs families is not shared among two of the three sequenced angiosperm genomes (Arabidopsis, poplar, and rice), suggesting that these miRNAs have recently evolved. miRNAs and their target genes have been conserved since the last common ancestor of bryophytes and seed plants more than 400 million years ago (Floyd and Bowman, 2004). Among the known families of miRNAs in Arabidopsis, 4 are conserved down to mosses, 20 are shared between Arabidopsis and rice, while 22 are conserved between Arabidopsis and poplar. The remaining families are so far unique to Arabidopsis, but as the genomes of species closely related to Arabidopsis become available, some of these families may be found to be common to these related species. Evolutionarily "young" miRNAs are predominantly found at single loci in the genome. The great majority of plant miRNAs genes are located in intergenic regions (Kim, 2005). Plant miRNAs genes are usually not arranged in tandem in the genome or co-expressed. Merchan et al. (2009) reported the existence of plant polycistronic precursors which contain non-homologous miRNAs that target transcripts encoding functionally related proteins. Deep sequencing of sRNAs under normal and various stress conditions will be necessary to uncover the full complement of miRNAs genes in any plant species. To understand the evolutionary history of miRNAs in any species, it will also be necessary to obtain the full complements of miRNAs genes in a number of species closely related to the species in question. Finally, uncovering miRNAs from key representative species spanning the entire evolutionary distance from unicellular plants to angiosperms will be necessary to provide a comprehensive picture of miRNAs evolution in plants. Due to the existence, at the time, of at least ~21 conserved miRNA families in higher plants, Sunkar and Jagadeeswaran (2008) conducted a homology based search using databases to identify orthologs or paralogs of the conserved miRNAs in large number of diverse plant. This contributed to understanding the evolution of miRNAs and miRNA-targeted gene regulations. They also reported the conservation of 6 newly found Arabidopsis miRNA homologs (miR158, miR391, miR824, miR825, miR827 and miR840) and 2 small RNAs (small-85 and small-87) in Brassica spp.

Identification of plant miRNAs

The determination of the individual developmental roles of several plant miRNAs has been assisted by genetic screening. For example, miR319/JAW was discovered in a developmental screen of activation-tagged *A. thaliana* lines (Palatnik et al., 2003). Initially, miRNAs were identified by genetic screening. The application of this method was limited because it is expensive, time consuming, and dominated by chance (Lee et al., 1993; Wightman et al., 1993). To overcome some of the shortcomings of genetic screening, another experimental approach was recently described for isolating and identifying new miRNAs. This approach involves direct cloning after isolation of small RNAs (Lu et al., 2005; Fu et al., 2005). Small RNA molecules are first isolated by size fractionation and ligated to RNA adapters at their 5′ and 3′ ends. Finally, they are reverse transcribed into cDNA, which is then amplified and sequenced (Lu et al., 2005). Because only small RNAs are isolated and screened by this method, it is a more efficient way to obtain miRNAs than general genetic screening. Lu et al. (2005) further refined this method by combining it with massively parallel signature sequencing (MPSS) to study Arabidopsis miRNAs. This method can also quantify miRNAs abundance at the same time. However, the quantification of mature miRNAs is rather difficult due to their often low-abundance, short length, homology between miRNA species, and the inclusion of the mature miRNA sequence in the primary miRNA (primiRNA) and precursor miRNA (pre-miRNA) transcripts (Jensen et al., 2011). The third approach is the computational. Bioinformatics methods have been valuable in the identification of many plant miRNAs. However, these approaches identify mainly conserved miRNAs. Besides, some non-conserved miRNAs, mostly considered recently evolved miRNAs, have been discovered and appear to be species-specific (Allen et al., 2005; Felippes et al., 2008). Until recently, most sequence information such as ESTs or genome survey sequences (GSS) used for computational prediction of miRNAs was generated by the traditional Sanger sequencing method. Compared to conserved miRNAs, non-conserved miRNAs are often expressed at lower levels which make their detection more daunting using small-scale sequencing (Amiteye et al., 2011). Pyro sequencing methods now provide a rapid way to identify and profile small RNA populations in different plants, mutants, tissues, and at different stages of development. The development of high-throughput 454 pyrosequencing technology has therefore allowed the discovery of several non-conserved or less expressed miRNAs through cloning and deep sequencing of small RNA and transcriptome libraries in Arabidopsis (Rajagopalan et al., 2006; Fahlgren et al., 2007), wheat (Yao et al., 2007), tomato (Moxon et al., 2008). Star sequences of miRNAs (miRNAs*) are difficult to

Table 1. A summarized comparison of different approaches utilized in identifying miRNAs (extracted from Zang et al., 2006).

	Genetic screening	Direct cloning after isolation of small RNAs	Computational method	EST analysis
Specific software	No	No	Yes	No
Require genome sequence	No	No	Yes	No
Cost	High	High, but less than genetic screening	Moderate	Low
Efficiency	Low	High	Low	High
False positive possibility	Low	Low	High	Moderate
Need experimental confirmation	No	No	Yes	Moderate
Possibility for new miRNAs	High	High	High	Low
Suitable for a wide variety of species	Yes	Yes	No	Yes
Comprehensive	Yes	Yes	Moderate	Yes
MiRNA quantitative information	No	Yes	No	Somewhat

detect by conventional methods due to their rapid turnover. However, high throughput sequencing retrieved many of them and revealed their relative abundance in different organism (Soares et al., 2009). Deep Sequencing was utilized by Tang et al. (2012) to identify sRNA-mediated responses to cold stress in a temperature-sensitive wheat line. Several algorithms are currently available for prediction of putative miRNAs-mRNA targets in plants (Hofacker, 2003; Zuker, 2003). One of the most widely employed miRNAs target finding software is the miRU (Zhang, 2005). The miRU system searches for target sites from selected databases for potential complementary target sites in miRNAs-target recognition with acceptable mismatches. It requires the input of a mature miRNAs sequence followed by selection of the dataset for prediction of mRNA target in the organism of interest. The fourth approach is the Expressed Sequence Tag (EST) analysis. Identification and characterization of plant miRNAs are usually done by expressed sequence tags (ESTs) analyses and various computational approaches (Zang et al. 2005). Expressed sequence tags (ESTs) are partial cDNA sequences of expressed genes cloned into a plasmid. EST analysis has proven to be an economically feasible alternative for gene discovery in species lacking a draft genome sequence, and many important genes have been found through EST analysis. This is feasible because miRNAs are generated from long precursor hairpin structures that can be found by searching ESTs. The strategy is to search for sequences containing conserved mature miRNAs and check if these miRNAs bearing ESTs can form stable secondary stem-loop structures. Computational approaches involve scanning several EST databases and performing similarity searches using BLASTn. EST sequences which closely matched (n/n, n-1/n and n-2/n nucleotide matches, where n equals the previously known Arabidopsis miRNAs length) the previously known Arabidopsis miRNAs can be chosen, and their secondary structures can be predicted and generated using the Zucker folding algorithm with mFold (Mathews et al., 1999; Zuker, 2003). The secondary structure of hairpin stem-loop, helicity of the miRNAs and energy should be assigned a score. If there is more than one hairpin stem-loop structure for the ESTs containing the miRNAs, each is scored and the hairpin structure with the highest score shall be considered the miRNAs hairpin stem-loop structure. Closely related EST sequences can be blasted against each other and analyzed. If the ESTs have a high similarity (E value less than e-100), it indicated that these ESTs were created from the same mRNA, and can then be considered as one novel miRNAs (Zuker, 2003). In the EST database, ESTs were unequally obtained from different tissues. Some tissues may contribute more ESTs than other tissues. Thus, more

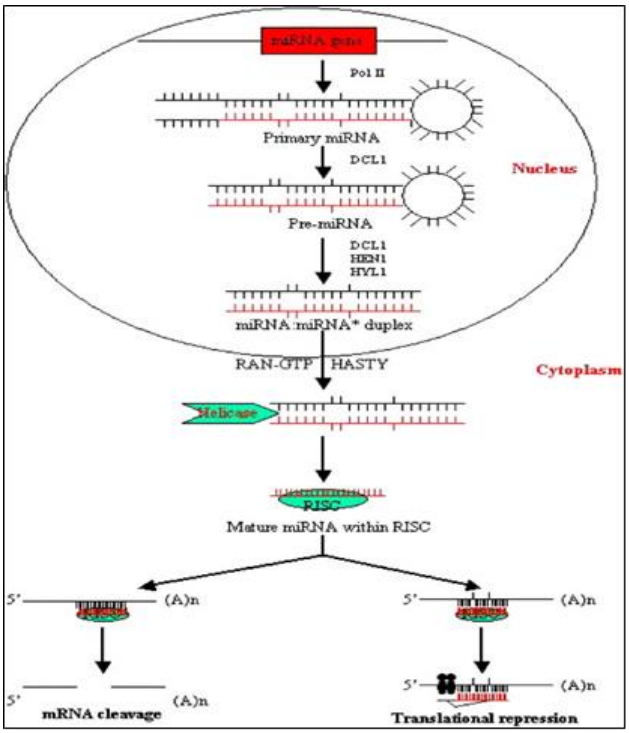

Fig 1. miRNAs Biogenesis **(**Depicted from Zhang, 2005).

experiments need to be conducted to confirm this conclusion. However, this approach gave more clues to study plant microRNAs, and this strategy alleviates the usually difficult step of predicting the correct tissues and conditions to search for expression evidence in a directed manner. Target-align are the latest tool for predicting miRNAs targets in plants. It exhibits strong sensitivity and accuracy for identifying miRNAs targets (Xie and Zang, 2010). Polysome associated RNA (psRNA) target is a plant small RNA analysis server, which is used for accurate plant miRNAs target prediction, was developed by Zhao Bioinformatics Laboratory Ardmore, UK (http://www.plantgrn.org/psRNATarget). Prediction methods have made it easy to identify potential miRNAs and their targets. Smalheiser (2003) and Zhang (2005) successfully identified miRNAs by mining the repository of available ESTs. Bartel (2004) used Arabidopsis microRNA against an EST database to search for potential evidence of miRNAs in other plant species. A comparison of different approaches of miRNAs identification is summarized in Table (1). Jensen et al. (2011) compared two commercial global

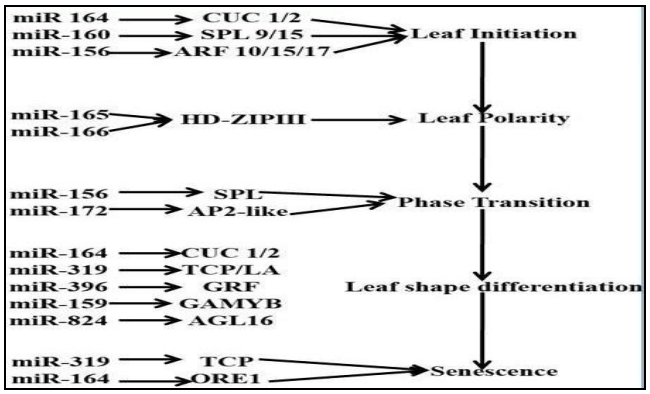

Fig 2. Schematic representation of miRNAs responsible for the leaf developmental stages.

miRNA expression profiling platforms for detection of less abundant miRNAs, namely the TaqMan and miRCURY the mercury platform proved better due to its better sensitivity and linearity in the low miRNA concentration range. Wang et al. (2012) conducted a comparative miRNAome analysis which revealed seven fibers initiation-related and 36 new miRNAs in developing cotton ovules. And, recently Shao et al. (2012) reported a reversed framework for the identification of microRNA-target pairs in plants.

Biogenesis

Bartel (2004), Kurihara and Watanabe (2004), Lee et al. (2002) and Zhang (2005) showed that microRNA biogenesis requires multiple steps in order to form mature miRNAs from miRNAs genes (Fig. 1). First, a miRNAs gene is transcribed to a primary miRNAs (pri-miRNA), which is usually a long sequence of more than several hundred nucleotides. Like transcription of most protein-coding genes, the miRNAs transcription is governed by RNA polymerase II (Pol II) enzymes, and the pri miRNAs are 5′ -capped and 3′-polyadenylated (Lee et al., 2004; Bartel, 2004; Kurihara and Watanabe, 2004; Xie et al., 2005). Bioinformatics analysis of the sequence regions upstream of the transcription start sites of the miRNAs genes identified putative binding motifs for a number of known transcription factors (Megraw et al., 2006). Second, the pri-miRNA is cleaved to a stem loop intermediate called miRNAs precursor or pre-miRNAs. This step is controlled by the enzyme Dicer like 1 (DCL1) in plants (Kurihara and Watanabe, 2004; Tang et al., 2003). Plant miRNAs are cleaved into miRNAs: miRNAs* duplex possibly by DCL1 in the nucleus (Papp et al., 2003; Bartel, 2004) and is assisted by two other proteins, hyponasty leaves1 (HYL1) and serrate (SE). HYL1 belongs to a family of double-stranded RNA (dsRNA) binding protein and interacts with DCL1 *in vitro* and *in vivo* (Lu and Fedoroff 2000; Hiraguri et al., 2005; Kurihara et al., 2006). SE, a C2H2 zinc finger protein, interacts with DCL1 and HYL1 and plays a role in the processing of pri-miRNAs to precursor-miRNAs (Pre-miRNAs) (Lobbes et al., 2006; Yang et al., 2006). After the miRNAs/miRNAs* duplexes are released from the pre-miRNAs by the DCL1 activities, they are methylated at the 2′ OH of the 3′-ends by HUA enhancer1 (*HEN1*), a small RNA methyl transferase (Yu et al., 2005; Yang et al., 2006). This enzyme has two dsRNA-binding domains and a nuclear localization signal. The methylation of the miRNAs/miRNAs* duplex has been shown to be required to protect miRNAs against the 3′-end uridylation activity and subsequent degradation (Li et al., 2005). The duplex is then translocated into the cytoplasm by nucleocytoplasmic transporter 1 (HASTY) (Park et al., 2002). In the cytoplasm, miRNAs are converted into single strand mature miRNAs by helicases (Bartel, 2004). The methylated miRNAs/miRNAs* duplexes undergo RNA Induced Silencing Complexes RISCs assembly. RNA interference is mediated by a family of ribonucleoprotein complexes called RNA-induced silencing complexes (RISCs), which can be programmed to target virtually any nucleic acid sequence for silencing. The ability of RISC to locate target RNAs has been co-opted by evolution many times to generate a broad spectrum of gene-silencing pathways. In this process, the miRNAs of the duplexes is selectively incorporated into the RISC, and the miRNAs* is removed and subsequently degraded (Hammond et al., 2000; Hutvagner and Zamore 2002; and Schwarz et al., 2003). Finally, the mature miRNAs enter a ribonucleoprotein complex known as the RNA-induced silencing complex (RISC) which contains Argonaut proteins as the core components, (Hammond et al., 2000) and regulate targeted gene expression (Bartel, 2004). microRNA biogenesis is under feedback regulation such that the genes involved in miRNAs biogenesis and function are themselves regulated by miRNAs. The Dicer-Like 1 (*DCL1*) gene may be regulated by the status of miRNAs biogenesis by two different mechanisms. First, *DCL1* mRNA has a binding site for miR162, which leads to the cleavage of *DCL1* mRNA (Xie et al., 2003). Consistent with this, *DCL1* mRNA levels are elevated in the *hen1* mutant, in which the abundance of miR162 is reduced. Second, the 14th intron of the *DCL1* gene appears to harbor the precursor to miR838. The *AGO1* (Argonaut) gene encoding the main miRISC component is also under the regulation by a miRNAs. miR168 has a binding site in *AGO1* mRNA and leads to *AGO1*-mediated cleavage of *AGO1* mRNA (Vaucheret et al., 2004). Therefore, the amount of functional miR168 bound by *AGO1* determines the levels of *AGO1* mRNA. In addition, miR168 and *AGO1* genes are transcribed in a similar pattern, which probably ensures that *AGO1* is under the regulation of miR168 at all times and in all the cells that express *AGO1*.

Small RNA metabolism

MicroRNAs are known to target genes involved in the metabolism of small RNAs such as miRNAs and small interfering RNA (siRNA), RNA molecules of short length, or function. DCL1 contains a binding site for miR162, the binding of which leads to decreased DCL1 mRNA levels (Xie et al., 2003). The AGO1 gene that codes for a RISC component is targeted by miR168 (Vaucheret et al., 2004). AGO2, another Argonaut gene, contains a binding site for miR403 in its 3'UTR (Allen et al., 2005). The regulation of small RNA machinery by small RNAs indicates that the regulation may be under a feedback control.

Functions of plant miRNAs

Plant development leads to numerous cell, tissue and organ types. The detailed mechanisms governing organogenesis, particularly in plants, remain largely elusive. One way to approach organogenesis at the functional level is to genetically dissect the process and to study systematically a large number of mutants. The primary growth of a plant is the result of several processes that can be grouped into two distinct, but co-ordinated morphogenetic events: organogenesis and extension (Champagnat et al., 1986)**.** The roots develop along a different path giving rise to many lateral roots and a main root which develops into stele. Stele

can differentiate into xylem, cambium, phloem, cortex and rhizodermis. Several experiments have demonstrated that many miRNAs regulate various plant development processes, including leaf morphogenesis and polarity (Emery et al., 2003; Mallory et al., 2004; Juarez et al., 2004; Zhong and Ye, 2004; Bowman, 2004; Bao et al., 2004; Kim et al., 2005), floral differentiation and development (Aukerman and Sakai, 2003; Chen et al., 2004), root initiation and development (Laufs et al., 2004; Mallory et al., 2005; Guo et al., 2005), vascular development (Floyd and Bowman, 2004; Kim et al., 2005) and transition of plant growth from vegetative growth to reproductive growth (Floyd and Bowman, 2004; Achard et al., 2004). A majority of these miRNAs affect plant traits by regulating the expression of transcription factors that influence cell fate determination (Mallory et al., 2005; Lauter et al., 2005). Mishra and Mukherjee (2007) discussed this mechanism indicating how miRNAs genes are transcribed in response to an extracellular or hormonal signal received by Transport Inhibitor Response 1 (TIR1) protein located in the cell membrane. TIR1 then signals miRNAs biogenesis to begin transcribing miR164, miR171, miR156, miR159, miR165/166, miR-172 and miR319, and down regulation of Nitrogen Assimilation Control protein (*NAC*), Scare Crow Like (*SCL*), Squamosa Promoter Binding Protein Like (*SPL*), Apelate 2 (*AP2*), Homeobox-lucin zipper protein (*HD ZIP*) and *TCP* genes respectively, each affecting different cellular processes. This process is summarized in Fig. (2). TIR1 transcripts are targets of miR393, a conserved miRNA. miR393 down regulates TIR 1 (Sunkar and Zhu, 2004; Si-Ammour et al., 2011). All the above mentioned miRNAs are involved in one of the several processes involved in plant growth, development and reproduction. Recently, Zhang et al. (2012) identified 11 conserved miRNAs families including 17 miRNAs, their targets and stage-specific modulation during somatic embryogenesis of Japanese larch (*Larix leptolepis*), a gymnosperm, in a genome-wide search of microRNAs. The following is a brief account of some of these processes.

MicroRNAs in leaf development

Leaf development is a multifaceted process during which a small group of undifferentiated cells recruited in meristems will give rise to a flat structure organized into different cell types. During this developmental sequence, patterning, growth, and differentiation have to be tightly coordinated and intricate regulatory networks involving transcription factors and hormones are at play. Several experiments have demonstrated the transcription factors that control the asymmetry pattern along the adaxial/abaxial (upper/lower) axis in leaves. Phabulosa (*PHB*), phavoluta (*PHV*) and revoluta (*REV)* are the targets of miR165 and miR166, and are regulated by these two miRNAs. *PHB* is involved in abaxial leaf fates into adaxial leaf fates. REV regulates meristems initiation at lateral positions. *PHV* has overlapping functions with *PHB* and *REV* in patterning the apical portion of the embryo. *PHB, PHV* and *REV* encode members of *HD-ZIP* family which contains homeodomain-leucine zipper domains. Mallory et al., (2004) proved that dominant mutations in *PHB* and *PHV* map to a miR165/166 complementary site and impair miRNAs-guided cleavage of these mRNAs in vitro. All evidence shows that any mismatched pairing of miRNAs and target can cause dysregulation of the targets. This can confirm that disrupted miRNAs pairing causes the developmental defects in *PHB-d* mutants. And, that it does not happen because of the changes in PHB protein sequence but due to a change in the complementary binding pattern (Juarez et al., 2004; Zhong and Ye 2004; Bowman, 2004; Bao et al., 2004; Kim et al., 2005). microRNA156 regulates plastochron length (the time between the initiation of two successive leaves, the inverse of the leaf initiation rate) by quantitatively modulating the levels of Squamosa Promoter Binding Protein Like (*SPL)* transcription factors (Schwarz et al., 2008; Wang et al., 2008). The specific developmental roles of miR156, miR159, miR160, miR164, miR165/166, miR167, miR172, and miR319 in phase changes, leaf morphogenesis and polarity, root initiation, vascular development, transition from vegetative to reproductive growth, or floral differentiation have been described by Chen (2004) and Zhang et al., (2006). Cup Shaped Cotyledon (*CUC1*), *CUC2*, and *CUC3* define the boundary domain around organs in the *A. thaliana* meristems. *CUC1* and *CUC2* transcripts are targeted by microRNA (miRNAs*)*, miR164, encoded by miR164a, b, and c. Mutations in the miR164A gene deepen serration of the leaf margin. By contrast, leaves of plants over expressing miR164 have smooth margins. Enhanced leaf serration was observed following the expression of and miR164-resistant *CUC2* but not of a miR164-resistant *CUC1* (Nikovics et al., 2006). In different organs during plant development, these Auxin response factors (ARFs) carry out specific and overlapping functions, which are all regulated by miR160 (Mallory et al., 2005). miR160 regulation of these ARF genes is necessary for proper phyllotaxis in the rosette. miR156 regulates plastochron length (the time between the initiation of two successive leaves, the inverse of the leaf initiation rate) by quantitatively modulating the levels of *SPL* (Squamosa Promoter Binding Protein-like) transcription factors (Schwarz et al., 2008; Wang et al., 2008). Over expression of miR156 accelerates the rate of leaf initiation. miRNAs 165 and 166 are able to cleave their target mRNAs of *HD-ZIP III* genes, thus regulating the functions of these genes. Thus miR165 and miR166 differentially regulate the functions of *HD-ZIP III* genes in Arabidopsis (Zhong and Zheng-Hua, 2007). Fig. 2 summarizes the role of miRNAs which are responsible for the leaf developmental stages like leaf initiation (miR164, miR160 and miR156), leaf polarity (miR165 and miR166), phase transition (miR156 and miR172), leaf shape differentiation (miR164, miR319, miR396, miR159 and miR824) and senescence (miR319 and miR164). These results were recently confirmed by Zhang et al. (2012), examining the roles of miRNA in somatic embryogenesis (SE). They reported that miR171a/b might exert function on proembryogenic masses (PEMs), while miR171c acts in the induction process of larch SE; miR397 and miR398 is mainly involved in modulation of PEM propagation and transition to single embryo. Furthermore, miR162 and miR168 were found to exert their regulatory function during total SE process, especially during stages 5–8. That study also suggested that miR156, miR159, miR160, miR166, miR167, and miR390 might play regulatory roles during cotyledonary embryo development.

microRNAs in floral development and vegetative phase change

The function of plant miRNAs was initially studied predominantly using transgenic approaches, in which mutations were introduced into the targets that rendered them insensitive to miRNAs action without changing the amino acid sequence of the encoded proteins (Palatnik et al., 2003; Chen, 2004). Floral initiation and floral organ development are both regulated by the phytohormone gibberellin (GA).

miRNAs function throughout flower development, from the earliest stages (floral induction) to very late stages (floral organ cell type specification). Flower development is now known to be regulated by miRNAs (Chen, 2004). Over expression of miR172 inhibited the translation of the Apetala 2 (*AP2*) gene and *AP2-like* genes and resulted in early flowering and disrupting the specification of floral organ identity. MicroRNA172 regulates *AP2* gene expression through translational inhibition rather than through mRNA cleavage (Aukerman et al., 2003; Chen, 2004). Three Scare Crow like (*SCL*) genes (*SCL6-II*, *SCL6-III* and *SCL6-IV*) in Arabidopsis and 4 in rice have perfect complementary sequences with miR171 (Reinhart et al., 2002; Llave, 2004). This indicates that these *SCL* genes are the targets of miR171. Another example is the inactivation of the miR164c locus, in the early extrapetals1 (*eep1*) mutant, affects petal number in early flowers (Baker et al., 2005). Several other microRNAs are also involved in floral development. . Recently, Gibberellin was found to regulate the Arabidopsis floral transition through miR156-targeted Squamosa promoter binding–like transcription factors (Yu et al., 2012). Shan et al. (2012) isolated and characterized six *NAC* genes, designated *MaNAC1–MaNAC6* from banana fruit. They suggested that *MaNACs* such as *MaNAC1*/*MaNAC2* interact with ethylene signaling components involved in banana fruit ripening.

microRNAs in shoot and root development

Five members of cup shaped cotyledon 1 (*CUC1*, *CUC2*, *NAM* (No Apical Meristems), *NAC1*, *At5g07680*, and *At5g61430*) of the NAC domain gene family in Arabidopsis were identified to have complementary sites with miR164 and are targets of miR164. Over expression of miR164 resulted in the fusion of vegetative and floral organs, unbalanced floral organ numbers, and reduced lateral root emergence (Rhoades et al., 2002; Mallory et al., 2004). microRNA164 also controlled organ boundaries and root formation by regulating *NAC1* and *CUC2* expression.

microRNAs in vascular development

Class III homeodomain-leucine zipper proteins regulate critical aspects of plant development, including lateral organ polarity, apical and lateral meristems formation, and vascular development. A member of this transcription factor family, homeobox-leucine zipper protein ATHB-15 (*ATHB15*), is exclusively expressed in vascular tissues (Baima et al., 2001). Recently, a miRNAs binding sequence has been identified in *ATHB15* mRNA, suggesting that a molecular mechanism governed by miRNAs binding may direct vascular development through *ATHB15*. It is a target for miR-166 (Kim et al., 2005). Over expression of miR166a resulted in decreasing *ATHB15* mRNA levels and caused accelerated vascular cell differentiation from cambial/procambial cells and consequently produced an altered vascular system with expanded xylem tissue and an inter-fascicular region (Kim et al., 2005). This regulation mechanism may exist in all vascular plant species (Floyd and Bowman, 2004; Kim et al., 2005). HD-ZIP also regulates vascular development as well as lateral organ polarity and meristems formation (Rhoades et al., 2002). Hu et al. (2012) identified the homeodomain-leucine zipper gene family in Poplar (*Populus trichocarpa*) and examined the evolutionary expansion and expression of their profile. Their findings indicated that that segmental duplications contributed significantly to the expansion of Populus HD-ZIP gene family, the exon/intron organization and conserved motif composition of this gene family in Populus is highly conservative in the same subfamily, and thus suggested that the members in the same subfamilies may also have conservative functionalities. They also reported that the analyses revealed that 89% (56 out of 63) of Populus HD-ZIPs were duplicate genes that might have been retained by substantial subfunctionalization.

Auxin signalling

Auxins have an essential role in coordination of many growth and behavioural processes in the plant's life cycle. Auxins mediate the degradation of a class of transcription repressors known as Aux/IAA* (Auxin/Indole acetic acid) proteins through the ubiquitin proteasome pathway. The Aux/IAA proteins heterodimerize with members of the auxin response factor (ARF) family of transcription activators and repressors and inhibit the activities of the activating ARFs. Auxin is bound by Transport Inhibitor Response1 (TIR1), an F-box protein in the ubiquitin protein ligase, SCFTIR1. The F-box domain is a protein structural motif of about 50 amino acids that mediates protein-protein interactions. It was first identified in cyclin F, a protein that controls the transition of different phases in the cell cycle. The F-box motif interacts directly with the Skp, Cullin, F-box containing complex (or SCF complex which is a multi-protein E3 ubiquitin ligase complex catalyzing the ubiquitination of proteins destined for proteasomal degradation) protein Skp1 (Bai et al.,1996; Baima et al., 1995) and F-box domains commonly exist in proteins in concert with other protein-protein interaction motifs such as leucine-rich repeats and WD repeats (tryptophan-aspartate repeat), a sequence motif approximately 31 amino acids long, that encodes a structural repeat (Orthologous to the *Saccharomyces cerevisiae* gene *MET30*, the *Drosophila melanogaster* gene *slmb* and the human gene *βTRCP*, all of which function as components of SCF ubiquitin-ligase complexes), which are thought to mediate interactions with SCF substrates (Kipreos et al., 2000). Auxin promotes the interaction between SCFTIR1 and its substrates, Aux/IAA proteins, to lead to their proteolytic degradation. A number of genes in auxin signalling are targets of miRNAs. The TIR1 auxin receptor is a target of miR393 (Bonnet et al., 2004; Rhoades et al., 2002; Wang et al., 2004; Adai et al., 2005). *NAC1*, which encodes transcription factor acting downstream of *TIR1* to promote lateral root formation, is a target of miR164 (Gou et al., 2005). Other plant growth regulators are also involved in plant responses to different biotic and abiotic stresses such as abscisic acid and ethylene. Recently, Bansal et al. (2011) discussed the Omics of abscisic acid. A comprehensive account of other aspects of the omics of the relationship between growth regulators and abiotic stresses have been given by Tuteja et al. (2011), which can't be discussed herein due to the limited space and objective of this review.

microRNAs in environmental stress

Plants are constantly subjected to various abiotic stresses which lead to morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang et al., 2001). Drought, salinity, extreme temperatures and oxidative stress are often interconnected inducing similar cellular damage exemplified by the fact that drought and/or salinization are manifested primarily as osmotic stress that results in disruption of homeostasis and ion distribution in the cell (Serrano et al., 1999; Zhu, 2001). Oxidative stress, *which* frequently accompanies high

temperature, salinity, or drought stress, may cause denaturation of functional and structural proteins (Smirnoff, 1998). As a result, these diverse environmental stresses often activate similar cell signaling pathways (Shinozaki and Yamaguchi-Shinozaki 1997; Knight and Knight 2001; Zhu 2001, 2002) and cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants and accumulation of compatible solutes (Vierling, 1991). The initial stress signals (e.g. osmotic and ionic effects, or temperature, membrane fluidity changes) initiate the downstream signaling process and transcription controls which activate stress-responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes. Inadequate response at one or several steps in the signaling and gene activation may ultimately result in irreversible changes of cellular homeostasis and in the destruction of functional and structural proteins and membranes, leading to cell death. miRNAs likely acquire new functions in regulating gene expression in response to these stresses. Studies showed that miR402 was over expressed during drought, cold, salinity and other environmental stresses for plants (Sunkar and Zhu, 2004). Two research groups independently found that either abscisic acid or gibberelic acid treatments regulated the miR159 expression (Sunkar and Zhu, 2004; Achard et al., 2004). Zhao et al., (2007) and reported that miR169g expression was upregulated in rice plants experiencing drought stress. miR393 is strongly upregulated by cold, dehydration, Sodium chloride (NaCl), and abscisic acid (ABA) treatments (Sunkar and Zhu, 2004). MiR397b show low but detectable expression in leaves, roots, and young seedlings but are undetectable in stems and inflorescence tissues. miR397b and miR402 are slightly upregulated by all the stress treatments, whereas miR319c appears to be upregulated by cold but not dehydration, sodium chloride (NaCl), or ABA whereas miR319c appears to be upregulated by cold but not dehydration, NaCl, or ABA. Interestingly, miR389a.1 appears to be downregulated by all of the stress treatments (Sunkar and Zhu, 2004). Reactive Oxygen Species (ROS) levels maintained primarily by Superoxide Dismutases (SODs) which exist in conjunction with several co-factors including, Cu-Zn, Fe, Ni, and Mn. Cu-Zn SODs are encoded by CSD1, CSD2, and CSD3 (Copper Dismutases in Arabidopsis thaliana (Sunkar et al., 2006). The miR398 was predicted to target CSD1 and CSD2 in 2004 (Bonnet et al., 2004; Rhoades and Bartel, 2004) and was confirmed later (Sunkar et al., 2006). Conserved miRNAs and their expression profile during salinity stress in cowpea (*Vigna unguiculata*) which is a salt sensitive member of legumes were studied using comparative genomic approach and strict filtering criteria. Eighteen conserved V. unguiculata miRNAs belonging to 16 distinct miRNAs families were identified. And, based on these sequences, fifteen potential target genes were predicted and identified as transcription factors. Seven of these predicted miRNAs were experimentally validated in root tissues and found up-regulated during salt stress as revealed by qRT-PCR. This study reported that perfectly cleaved Auxin response factor (ARF), the target transcript of V. unguiculata miR160 was detected successfully by modified 5′ RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE) method (Paul et al., 2011). The improvement of abiotic stress tolerance of agricultural plants can only be achieved, practically, by combining traditional and molecular breeding (Kasuga et al., 1999; Dunwell, 2000; Wang et al., 2001). Thus, a comprehensive breeding strategy for abiotic stress tolerance may include the following steps and approaches: (i) conventional breeding and germplasm selection, especially of wild relevant species; (ii) elucidation of the specific molecular control mechanisms in tolerant and sensitive genotypes; (iii) biotechnology-oriented improvement of selection and breeding procedures through functional genomics analysis, use of molecular probes and markers for selection among natural and bred populations, and transformation with specific genes; and (iv) improvement and adaptation of current agricultural practices (Wang et al., 2003). Microarray data can be used to analyze and integrate changes in metabolic pathways due to stress and address the consequent changes in gene expression. Microarray results of the Arabidopsis transcriptome indicate that several genes could be upregulated during multiple stresses. A significant increase in the GC content of stress regulated miRNAs sequences was observed proving that miRNAs act as ubiquitous regulators under stress conditions. GC content may also be considered as a critical parameter for predicting stress regulated miRNAs in plants like Arabidopsis thaliana (Mishra et al., 2009). Crop resistance to abiotic stress and the applications of plant omics for their improvement has been extensively examined by Tuteja et al. (2012). Barrera-Figueroa et al. (2012) identified new (70 miRNAs that are not present in the miRBase) and abiotic stress-regulated microRNAs in rice inflorescences utilizing high throughput sequencing. They identified 18, 15, and 10 miRNAs that were regulated by drought, cold and salt stress conditions, respectively.

miRNAs in plant virus and nematode infection/resistance

Helper component-proteinase (*HC-Pro*), *p19*, *p21*, and *p69* play important roles in the virus response to plant antiviral silencing response. These suppressor genes are usually called pathogenicity factors, and they cause disease and various developmental abnormalities (Chapman et al., 2004). *HC-Pro* decreased miRNAs levels, interfered with miR171 activity, and caused miR171- related developmental defects (Kasschau et al., 2003). Recently, (Dalzell et al., 2010) have shown that 21 bp siRNAs, specific to the gene encoding FMRFamide- like peptide (flp), are sufficient to silence the gene in infective stage juveniles (J2) of potato cyst nematode *Globodera pallida*, and root-knot nematode *Meloidogyne incognita*. (Charlton et al., 2010) showed that suppression of two *M. incognita* genes (dual oxidase and a subunit of a signal peptidase required for the processing of nematode secreted proteins) using RNA interference (RNAi) resulted in the reduction in the number of nematodes by 50%. Ibrahim et al. (2010) attempted to broaden resistance of soybean against the root-knot nematode *M. incognita* by silencing the genes encoding L-lactate dehydrogenase, mitochondrial stress-70 protein precursor, ATP synthase beta-chain mitochondrial precursor, and tyrosine phosphatase using RNAi gene silencing.

miRNAs Analysis in Date Palm and Desert Plants

Numerous plant genomes have been sequenced and searched for miRNAs, and the search is still going. Lu et al. (2008) conducted a genome-wide analysis of rice microRNAs which revealed the presence of natural antisense microRNAs, termed nat-miRNAs. Recently Schreiber et al. (2011) reported on miRNAs discovery in barley through deep sequencing of short reads. This adds to the high importance of the data generated from genome sequencing projects for different plant species. It would be of interest to search and compare the genome of rice as a water logging tolerant plant to that of date palm as a highly drought and salinity tolerant

plant, both are monocotyledonous, for miRNAs. Furthermore, Jojoba is a dicotyledonous desert plant with high economic value and high tolerance to salinity, drought, and pest stresses (Aly and Basarir, 2012). Including jojoba in such comparisons may further shed lights on miRNAs. Ramegowda et al. (2012) proved that transcription factor EcNAC1 from finger millet confers abiotic stress-tolerance in tobacco and concluded that genes from stress adapted species are functionally more efficient in improving stress tolerance. Furthermore, this also suggested that stress-adapted species might have novel candidate genes and/or unique mechanisms which can enhance efforts to improve genetic engineering of susceptible species for stress-tolerance. The genome draft of *Phoenix dactylifera* var. Khalas has been published by Weil Cornell Medical College in Qatar (Al-Dous et al., 2011). The date palm genome contains 18 pairs of chromosomes5 and our analysis suggests a genome size of ~658 Mb. The ~380 Mb sequence, spanning mainly gene-rich regions, includes >25,000 gene models and is predicted to cover ~90% of genes and ~60% of the genome. To produce the draft map, the WCMC-Q researchers used a next-generation sequencing approach. Also, the complete chloroplast (cp) genome sequence of date palm has been published (Yang et al., 2010; Khan et al., 2011). The major advantages of the cp genome are that it is maternally inherited, and its gene content is rather conserved in angiosperm. The objectives of this project were to examine date palm bioinformatics, genetics, biochemistry, chloroplast transcriptomes and post-genomics. Interestingly, date palm has a typical cp genome similar to that of tobacco (a dicot), with little rearrangement and gene loss or gain. Using the raw genomic information, we can make an approach to predict the putative miRNAs in the date palm genome using several bioinformatics approaches. Zhang (2005) made an approach to identify miRNAs using ESTs. Because the ESTs and expressed genes come from the production of true gene expression, this analysis can provide more evidence and confidence in the discovery of new potential miRNAs in date palm and their targeted genes. Previously known miRNAs sequences can be obtained from the miRNAs Registry Database. Alignments of the known plant miRNAs can be performed and a phylogenetic tree can be constructed. Utilizing the publicly available EST databases, *Phoenix dactylifera* nuclear and chloroplast genomes can be searched using Blastn (Altschul et al., 1997) by comparing all ESTs to all previously known mature miRNAs listed in the miRNAs Registry Database. A research project to study diversity and to partially sequence the nuclear genome of selected UAE cultivars is currently underway in the authors' labs in UAE in collaboration with USA labs (UAE University grant No. 31F002, Genomic tool development and full genome sequencing of date palm variety, Naghal. M. Aly, PI.). The data generated by the above mentioned groups and others will enable more facile fingerprinting, phylogeny, breeding studies as well as genomic sequence analysis which may provide more understanding of miRNAs role(s) in plant development.

Conclusions

Plant development is a tightly controlled process that is regulated at various levels of gene expression. The discovery of miRNAs has revealed a level of post transcriptional control that is important for fine-tuning the regulatory processes. Though miRNAs are very small in size, they seem to play a significant role in genome biology. The conserved patterns of miRNAs in plants, as orthologs or homologs lead to the identification of new miRNAs. Although traditional computational approaches have certain advantages and have made great progress in predicting new potential miRNAs, it is difficult to predict miRNAs in species with unsequenced genomes because these approaches are based on the availability of a genomic sequence. The majority of investigations on miRNAs were limited to the few model species with available genomic sequences, such as *C. elegans*, human, Arabidopsis, rice, etc. However, more plant species, e.g. wheat, sorghum, cotton, soybean, Brassica and others are being investigated now with the help of modern technologies and other high-throughput computational approaches. This will allow more accurate systematic analysis of genome data which will gradually reveal the panorama of the miRNAs and help us to further understand the evolutionary aspects, functions, structure, regulation of miRNAs and their target identification as well as construction of regulatory networks. Combined with bioinformatics and experimental approaches to separate miRNAs from short-interfering RNAs (siRNAs), the miRNA discovery pipeline is likely to accelerate. sRNAs may play an important role in the study of divergence and evolutionary analysis in the desert plant species like date palm (*Phoenix dactylifera* L.) and jojoba (*Simmondsia chinensis*) etc..., once their complete genomes sequences are reported. This may provide more knowledge related not only to developmental processes, but also to environmental stress biology. Furthermore, miRNAs may be of interest if utilized in improving plants though genetic engineering. Obvious choices are silencing genes involved in plant susceptibility to abiotic and biotic stresses, or silencing pathogenic genes. The search for miRNAs is advancing at high speed that it is obvious that no one review can cover a fully inclusive account for it. Furthermore, the expected outcomes may be more than one can speculate at this point.

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