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MICRORNAs AND THEIR REGULATORY ROLE IN PLANTS: A REVIEW

***KALASKAR, S. R., DHEMBRE, V. M., DAPKE, J. S., GAIKWAD, B. M., PATIL V. S., BILWAL, B. B., KANBI, V. H. AND ACHARYA, S.**

DEPARTMENT OF PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY
CHIMANBHAI PATEL COLLEGE OF AGRICULTURE
SARDARKRUSHINAGAR DANTIWADA AGRICULTURAL UNIVERSITY
SARDARKRUSHINAGAR – 385 506, GUJARAT, INDIA

E-MAIL: drsampatkalaskar@gmail.com

ABSTRACT

A powerful and unexpected role of microRNAs (miRNAs) in the control of plant growth by the silencing of native genes has been reported recently. MicroRNAs regulate almost every aspect of plant growth and development, including leaf morphogenesis and polarity, floral differentiation and development, root initiation and development, vascular development, and the transition from vegetative growth to reproductive growth. Even more intriguingly, it has been discovered that miRNAs play an important role in hormone signal transduction, the response to environmental stress, and pathogen invasion. These regulatory miRNAs stimulated the idea of developing artificial miRNAs that can silence specific gene(s). Such targeted gene silencing could permit the direct molecular modulation of plant traits, which could in turn be applied to the breeding of crop species. In this short review, the most recent advances are presented in understanding of the molecular machinery as well as the regulatory mechanisms involved in the expression of miRNAs in plants. Also, the current knowledge summarized for regarding the roles of miRNAs in various aspects of plant traits as well as the existing miRNA-based approaches to plant engineering.

KEY WORDS: Development, gene silencing, growth, miRNA, transcription

INTRODUCTION

Increasing world population, the scarcity of arable lands and water, global climate changes and the demand for bio-fuels as replacement energy source have led to serious concerns about the future sufficiency of the global production of food from crop plants. To overcome these problems, new agricultural technologies will be needed to ensure global food supply and security, in addition to water and land conservation efforts. In particular, crop improvements that confer tolerance to environmental stresses and soil viruses, and that provide high yield, will be required.

Recent studies have revealed powerful and unexpected roles of microRNAs (miRNAs) in the control of plant growth by the silencing of native genes. Although both of these have pivotal roles in gene silencing, the actions of miRNAs are more extensive and remarkable. MicroRNAs regulate almost every aspect of plant growth and development, including leaf morphogenesis and polarity, floral differentiation and development, root initiation and development, vascular development, and the transition from vegetative growth to reproductive growth. Even more intriguingly, it has been discovered that miRNAs play an important role in hormone signal transduction, the response to environmental stress, and pathogen invasion. These regulatory miRNAs stimulated the idea of developing artificial miRNAs that can silence specific gene(s). Such targeted gene silencing could permit the direct molecular modulation of plant traits, which could in turn be applied to the breeding of crop species. Here, we summarize current knowledge regarding the roles of miRNAs in various aspects of plant traits as well as the existing miRNA-based approaches to plant engineering. We also consider recent advances and the future challenges of putting these approaches to practical use in breeding programs for the purpose of increasing crop yield (Liu and Chen, 2010).

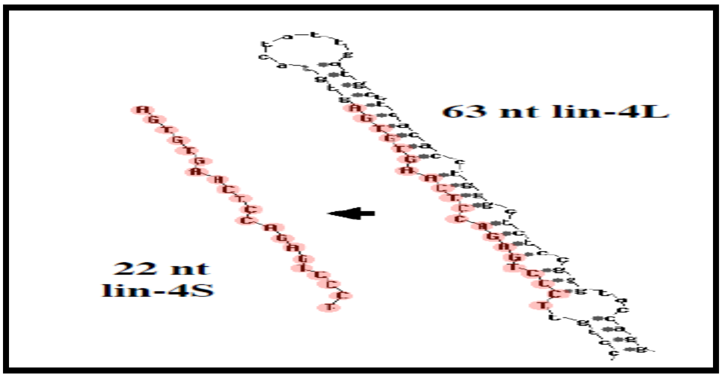
What is microRNA?

A microRNA (abbreviated miRNA) is a short ribonucleic acid (RNA) molecule found in eukaryotic cells. A microRNA molecule has very few nucleotides (an average of 22) compared with other RNAs. MicroRNAs are post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts (mRNAs), usually resulting in translational repression or target degradation and gene silencing (Bartel, 2004).

The first miRNAs were characterized in the early 1990s. However, miRNAs were not recognized as a distinct class of biological regulators with conserved functions until the early 2000s. Since then, miRNA research has revealed multiple roles in negative regulation (transcript degradation and sequestering, translational suppression) and possible involvement in positive regulation (transcriptional and translational activation). By affecting gene regulation, miRNAs are likely to be involved in most biological processes. Different sets of expressed miRNAs are found in different cell types and tissues.

Discovery

MicroRNAs were discovered in 1993 by Victor Ambros, Rosalind Lee and Rhonda Feinbaum during a study of the gene *lin-14* in *C. elegans* development (Lee et al., 1993). They found that LIN-14 protein abundance was regulated by a short RNA product encoded by the *lin-4* gene. A 63-nucleotide precursor from the *lin-4* gene matured to a 22-nucleotide RNA that contained sequences partially complementary to multiple sequences in the 3’ UTR of the *lin-14* mRNA.



This complementarily was both necessary and sufficient to inhibit the translation of the *lin-14* mRNA into the LIN-14 protein. Retrospectively, the *lin-4* small RNA was the first microRNA to be identified, though at the time, it was thought to be a nematode idiosyncrasy. Only in 2000 was a second RNA characterized: let-7, which repressed *lin-41*, *lin-14*, *lin-28*, *lin-42* and *daf-12* expression during developmental stage transitions in *C. elegans*. let-7 was soon found to be conserved in many species, indicating the existence of a wider phenomenon (Reinhart *et al.,* 2000).

Nomenclature

Under a standard nomenclature system, names are assigned to experimentally confirmed miRNAs before publication of their discovery. The prefix "mir" is followed by a dash and a number, the latter often indicating order of naming. For example, mir-123 was named and likely discovered prior to mir-456. The un-capitalized "mir-" refers to the pre-miRNA, while a capitalized "miR-" refers to the mature form. miRNAs with nearly identical sequences bar one or two nucleotides are annotated with an additional lower case letter. For example, miR-123a would be closely related to miR-123b. Pre-miRNAs that lead to 100 per cent identical mature miRNAs, but that are located at different places in the genome are indicated with an additional dash-number suffix. For example, the pre-miRNAs hsa-mir-194-1 and hsa-mir-194-2 lead to an identical mature miRNA (hsa-miR-194), but are located in different regions of the genome (Ambros *et al.,* 2003).

All known miRNA families that are conserved between more than one plant species are listed together with the number of genes identified in the sequenced genomes. Rice miRNA families that have orthologs in maize, but do not appear to have orthologs in the eudicots (*Arabidopsis* and Populus) are marked with an asterisk (Figure 1). Twenty four miRNA families identified are highly conserved between all three sequenced plant genomes: *Arabidopsis*, *Oryza sativa*, and *Populus trichocarpa* (Table 1). The following families contain miRNA genes annotated with more than one number: miR156 (miR156 and miR157), miR159/319 (miR159 and miR319), miR166 (miR165 and miR166), miR171 (miR170 and miR171), and miR390 (miR390 and miR391) (Jones-Rhoades *et al.,* 2006).

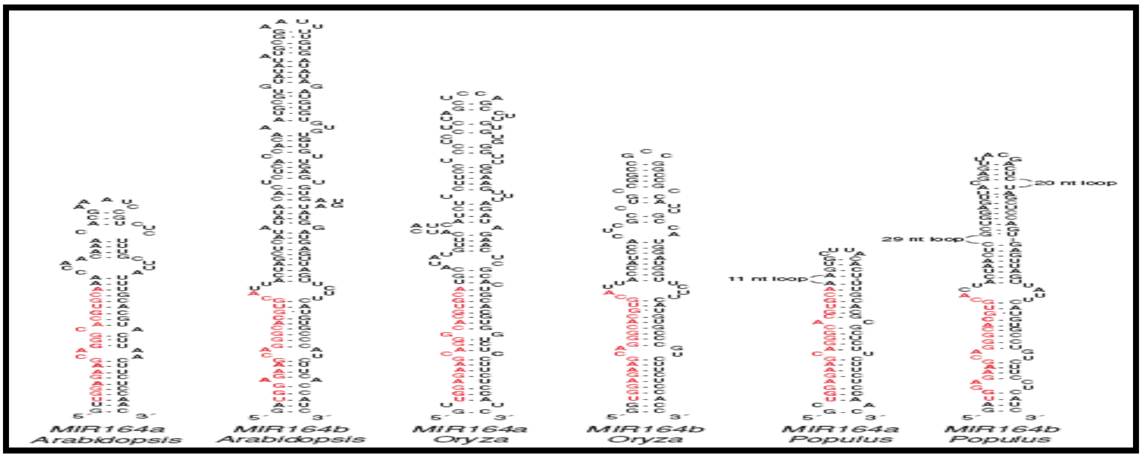


Fig. 1: Representative miR164 stem-loops from Arabidopsis, Oryza, and Populus. Segments corresponding to the mature miRNAs are shown in red.

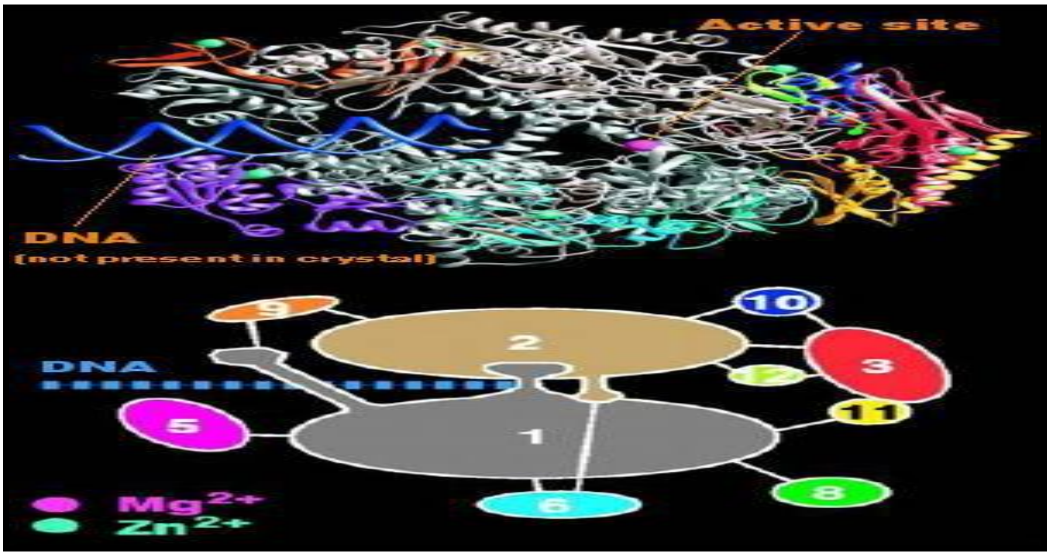
Biogenesis of plant microRNAs *(Figure 2)*

1. *Transcription of microRNA precursors*
2. *MicroRNA processing & export*
3. *MicroRNA incorporation into the silencing complex*

MicroRNA biogenesis requires multiple steps in order to form mature miRNAs from miRNA. First, a miRNA gene is transcribed to a primary miRNA (pri-miRNA), which is usually a long sequence of more than several hundred nucleotides. This step is controlled by Pol II enzymes.

RNA Pol II enzymes

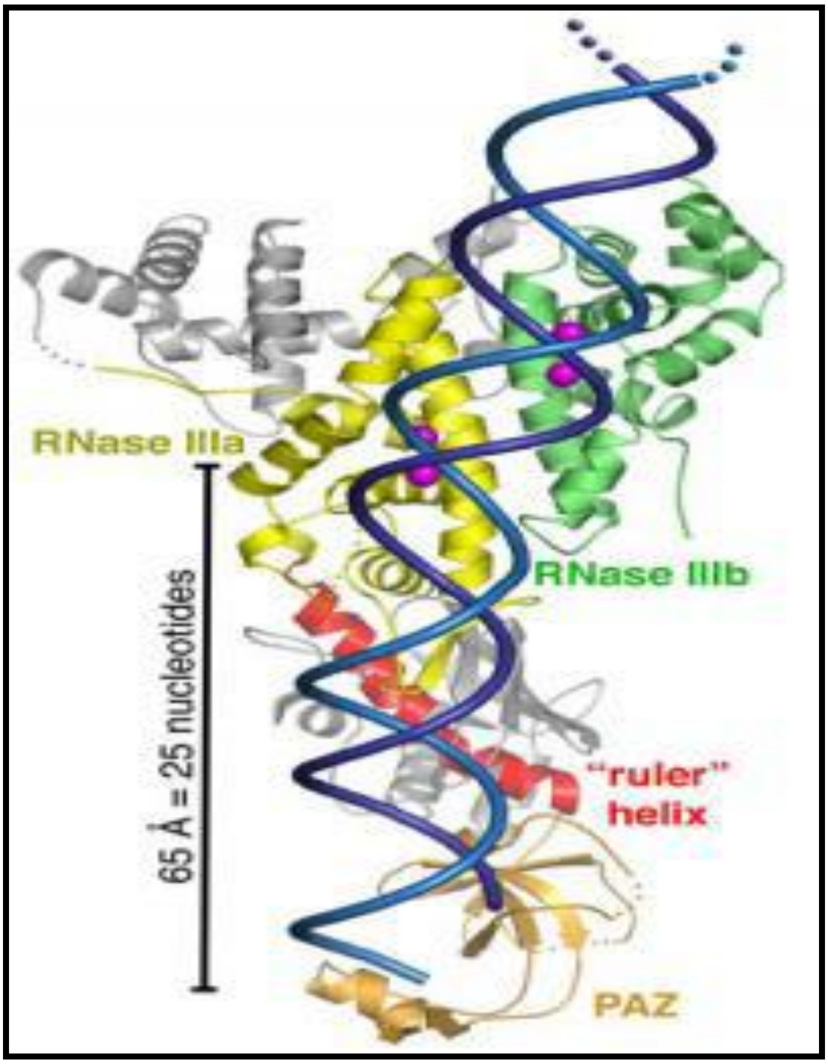
Pol II is responsible for the transcription of protein-coding genes, small nucleolar RNA genes and some small nuclear RNA genes. On the other hand, Pol III transcribes small non-coding RNAs including tRNAs, 5S rRNA, some snRNAs and other small RNAs. Several observations have suggested that Pol II may be the most important RNA polymerase engaged in miRNA gene transcription. First, the pri-miRNAs are longer than the ~70 nt pre-miRNAs and sometimes can be several thousand bases long. For example, pri-miR-21 is ~3433 nt in length, therefore, it is too long for Pol III- mediated transcription. Second, it is believed that the pri-miRNA gene sequences are flanked by promoters in the 5’ region that are able to transcribe heterologous mRNAs. Third, stretches with more than four U’s, which terminate the transcription of Pol III, widely exist in pri-miRNA sequences. Forth, human pri-miRNAs are polyadenylated and capped, which are the unique properties of pol II gene transcripts. However, some new evidence indicated that, in some cases, miRNAs can also be transcribed by Pol III.



Second, the pri-miRNA is cleaved to a stem loop intermediate called miRNA precursor or pre-miRNA. This step is controlled by the Drosha RNase III endonuclease in animals or by Dicer-like 1 enzyme (DCL1) in plants. Loss-of-function dcl1 mutants have low levels of miRNA Synthesis. In animals, pre-miRNAs are then transported by exportin 5 from the nucleus into the cytoplasm followed by formation of miRNA: miRNA* duplex and mature miRNAs by another RNase III-like enzyme called Dicer.

Dicer

Dicer, an RNase III endonuclease, which defines the other end of the mature MicroRNA. Dicer is an evolutionarily conserved enzyme in mammals, worms, fungi, flies and plants. It is composed of a helicase domain, a Piwi-Argonaute-Zwille (PAZ) domain, two RNase III motifs, a Domain of Unknown Function (DUF283) and a dsRNA binding domain (dsRBD). Dicer forms an intra-molecular dimer with its two RNase III domains, which generates an active catalytic site at the interface of the RNase III dimer for single dsRNA cleavage (Tang *et al.* 2003).



In this step, however, plant miRNAs differ from animals. Plant miRNAs are cleaved into miRNA: miRNA* duplex possibly by dicer like enzyme 1 (DCL1) in the nucleus rather than in the cytoplasm, then the duplex is translocated into the cytoplasm by HASTY, the plant orthologue of exportin 5). In the cytoplasm, both plant and animal miRNAs are unwound into single strand mature miRNAs by helicase. Finally, the mature miRNAs enter a ribonucleoprotein complex known as the RNA-induced silencing complex (RISC) (Hammond *et al.*, 2000), where they regulates the targeted gene expression (Bartel, 2004).

This suggests that miRNA biogenesis is complicated; several enzymes are required for processing long pri-miRNA to 20–24 nt mature miRNAs.

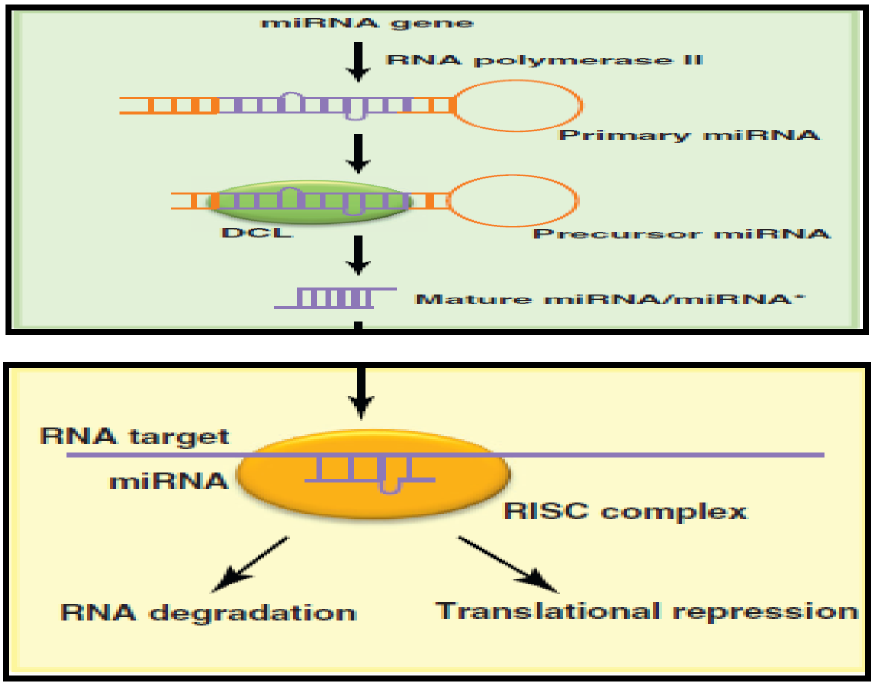


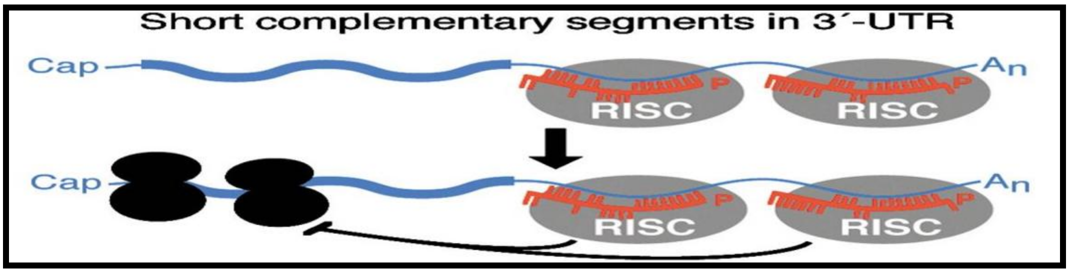
Fig. 2: Biogenesis of plant microRNAs

After pre-miRNA is processed into a miRNAmiRNA* duplex by Dicer, one of the RNA strands is incorporated into RISC for target recognition. RISC has strand bias and always incorporates the miRNA strand with the less stable 5' end in comparison with the miRNA* strand. The miRNA* strand is degraded in most cases. This strand bias may be the result of the relative ease of unwinding from one end of the duplex. A helicase likely carries out multiple trials from both ends in order to unwind the duplex. Occasionally, these trials lead to unwinding at the easier end of the complex, rendering a strong bias for the easier end. This is supported by the finding that in rare cases, where miRNAs have 5' ends with similar stability, both strands are incorporated into RISC (Hammond *et al.*, 2000).

Mechanism of mi RNA mediated gene regulation

1. Translational repression

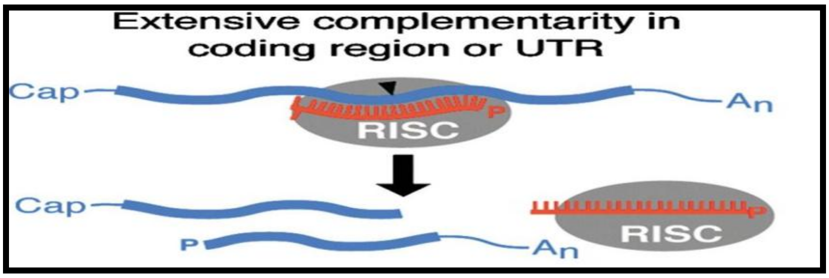
MiRNA-associated RISC can repress mRNA translation in a couple of ways. MicroRNAs in animals mostly suppress translation of their target mRNAs due to an imperfect base-pairing within 3' untranslated regions (UTRs). By binding to the 3' UTR of the mRNA, the miRNA has the ability to inhibit translation by directly interfering with translation initiation factors or by disrupting polyA tail function.



However, polyribosomes co-purify with RISC, indicating that RISC may also interrupt the continuation of translation by forming a stable complex with polyribosomes (Zeng *et al.*, 2002).

2. mRNA cleavage

The perfect base-pairing is thought to be the critical feature of miRNA-mediated mRNA cleavage, but it is not always sufficient in plants, suggesting the need for supplementary catalytic activity by RISC.



Regardless of the species, the perfect match between the miRNA and target mRNA is required for efficient cleavage, especially considering the precise location of the cut between residues 10 and 11 of the miRNA (Kasschau *et al.*, 2003).

3. mRNA degradation

MicroRNAs can also act upon their target mRNAs by increasing the rates of mRNA degradation in a RISC independent manner. By transfecting miR-1 and miR-124 into human cells and using microarray, Lim *et al.* (2003) showed that a significant number of mRNAs are down regulated in accordance with the miRNA expression. Another study reported that miR-16 contains an AU-rich sequence complementary to the AU-rich element (ARE) in the 3' un-translated region of unstable mRNAs, a sequence that is known to increase mRNA decay rates. The finding suggested that miRNAs are required for ARE-RNA turnover. Furthermore, Bagga *et al*. (2005) demonstrated that the expression of two of the earliest identified miRNAs, let-7 and lin-4, significantly decreased the levels of their target mRNAs.

Regulatory role of miRNA in plant growth and development

Although miRNAs are relatively small, they play an important role in gene expression. miRNA is considered as one of the most important post-transcriptional gene regulators, since it was originally recognized in 2001. In animals, miRNAs have proven to be involved in many functional processes such as development, stress response, and carcinogenesis. Although investigations of plant miRNAs are behind animal miRNAs, the versatile functions of plant miRNAs are also becoming clearer. A majority of miRNA functions are currently investigated by over expression or lowered expression of miRNAs. The following section summarizes several miRNA functions in plants.

Dicer-like enzyme 1 (DCL1) is an important enzyme that processes pri-miRNA to pre-miRNA then to miRNA: miRNA* duplex. Normal expression of the dcl1 gene is essential to synthesize and accumulate mature miRNA. Loss-of-function of the dcl1 gene reduced the level of mature miRNA and consequently caused many developmental abnormalities, such as altered leaf shape, delayed floral transition, arrested embryos at early stages, and female sterility. HASTY is an orthologue of exportin 5, which is the protein transporting miRNA: miRNA* duplex from the nucleus to the cytoplasm in *Arabidopsis*. Without HASTY, it is impossible to synthesize mature miRNAs in *Arabidopsis*. Loss-of-function of HASTY gene caused many pleiotropic phenotypes, including disrupting leaf shape and flower morphology, reducing fertility and accelerating vegetative phase change and disrupting the phyllotaxis of the inflorescence. These findings indicated that miRNAs are involved in a variety of developmental processes in plants. Several experiments have demonstrated that many miRNAs regulated various plant development processes, including leaf morphogenesis and polarity, floral differentiation and development, root initiation and development, vascular development, and transition of plant growth from vegetative growth to reproductive. A majority of these miRNAs affected the plant traits by regulating the expression of transcription factors that influence cell fate determination. At present, about 50 per cemt of the miRNA targets that have been identified are transcription factors. Of these miRNAs, at least 12 miRNA families target mRNAs encoding transcriptional factors that regulated plant development. Combined, these studies have illustrated that a majority of miRNAs regulated the plant development by controlling the levels of transcription factors that are important in development. Detailed effects of miRNA on different tissues are discussed in the following sections.

miRNA and leaf development

Plant leaves exhibit an asymmetry pattern along the adaxial/abaxial (upper/lower) axis. This pattern is controlled by the polarized expression homeodomain leucine zipper (HD-ZIP) and TCP transcription factor genes. Recently, several experiments demonstrated that all of these transcription factors are the targets miR166 and miR165. MicroR159/JAW also regulates the leaf development by targeting a subset of TCP transcription factor genes. Over expression of miRJAW resulted in low levels of all tested TCP mRNAs and caused jaw-D phenotypes, including uneven leaf shape and curvature. In contrast, over expression of miRJAW-resistant TCP mutants indicated that miRJAW-guided mRNA cleavage was sufficient to restrict TCP function. miR165/166 and miR159/JAW are essential for controlling the pattern and development of leaves by direct regulation of two classes of transcription factor genes (HD-ZIP and TCP) (Palatnik *et al.*, 2003).

Leaf shape and pattern is very important because is regulated by genetic, developmental, and environmental factors. The CUC genes belong to the NAC gene family, one of the largest families of plant-specific transcription factors, with 105 members in *Arabidopsis* and 75 members in rice. The *Arabidopsis* CUC1, CUC2, and CUC3 genes are involved in establishment of the embryonic meristem and determination of the boundary domain CUP-SHAPED COTYLEDON1 (CUC1), CUC2, and CUC3 defined the boundary domain around organs in the *Arabidopsis thaliana* meristem. CUC1 and CUC2 transcripts are targeted by a miRNA, miR164, encoded by MIR164A, B, and C. It showed that each MIR164 is transcribed to generate a large population of primary miRNAs of variable size with a locally conserved secondary structure around the pre-miRNA. It identified the mutations in the MIR164A gene that 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 a miR164-resistant CUC2, but not of a miR164-resistant CUC1. Furthermore, CUC2 inactivation abolished serration in mir164a mutants and the wild type, whereas CUC1 inactivation did not. Thus, CUC2 specifically controls leaf margin development. CUC2 and MIR164A are transcribed in overlapping domains at the margins of young leaf primordia, with transcription gradually restricted to the sinus, where the leaf margins become serrated. It suggested that leaf margin development is controlled by a two-step process in *Arabidopsis*. The pattern of serration is determined first, independently of CUC2 and miR164. The balance between co-expressed CUC2 and MIR164A then determines the extent of serration (Nikovics *et al.*, 2006).

MicroRNA miR156 is one of the most conserved and highly expressed miRNAs in plants. It has been found in moss, monocotyledons, and dicotyledons. The miR156 targets SQUAMOSA-promoter binding-like (SPL) transcription factor genes in plants.

In *Arabidopsis*, maize (*Zea mays*), and rice (*Oryza sativa*), over expressing miR156 resulted in dramatic morphologic changes, suggesting that miR156 has global regulatory function in plant development. In this study, it was found that the miR156 level is increased gradually over time in developing adult leaves (Xie *et al.*, 2012).

miRNA and floral development

Floral initiation and floral organ development are both regulated by the phytohormone gibberellin (GA). For example, in short-day photoperiods, the *Arabidopsis* floral transition is strongly promoted by GA-mediated activation of the floral meristem-identity gene LEAFY. GAMYB is a positive regulator of LEAFY, which is an important factor in floral development. GAMYB is one target of miR159. Study conducted by Achard *et al.*, 2004 indicated that miR159 directly guides the cleavage of mRNA encoding GAMYB-related proteins and further affects the GA-promoted activation of LEAFY. Over expression of miR159 resulted in a reduction of LEAFY transcript levels and further perturbed anther development and delayed flowering in short-day photoperiods). This indicated that miR159-guided GAMYB cleavage regulates plant anther development and flowering time.

Flower development is one of the most important stages of plant development, especially for flowering plants. Mature flowers consist of carpels, stamens, petals, and sepals. The origin of the flower can be well explained by the ABC model. According to this model, four flower organs are controlled by the combinatorial actions of three classes (A, B, and C) of transcription factors. APETALA 2 (AP2) is one of the classes A genes that play an important role in flowering time and flower morphology. AP2 and AP2-like proteins are required for plant flowering and floral organ identity.

Recently, two independent laboratories have demonstrated that AP2 is one of the targets of miR172.

Over expression of miR172 inhibited the translation of the AP2 gene and AP2-like genes (target of eat 1, TOE1) and resulted in early flowering and disrupting the specification of floral organ identity similar to loss-of function ap2 gene mutants. miR172 regulates ap2 gene expression through translational inhibition rather than through mRNA cleavage (Chen , 2004).

The ABC model of flower development explains how three classes of homeotic genes confer identity to the four types of floral organs. In *Arabidopsis thaliana*, APETALA2 (AP2) and AGAMOUS (AG) represent A- and C-class genes that act in an antagonistic fashion to specify perianth and reproductive organs, respectively. An apparent paradox was the finding that AP2 mRNA is supposedly uniformly distributed throughout young floral primordia. Although miR172 has a role in preventing AP2 protein accumulation, miR172 was reported to disappear from the periphery only several days after AG activation in the centre of the flower. Here, It was resolved that the enigmatic behaviour of AP2 and its negative regulator miR172 through careful expression analyses. It was also found that AP2 mRNA accumulates predominantly in the outer floral whorls, as expected for an A-class homeotic gene.

Its pattern overlaps only transiently with that of miR172, which found to be restricted to the centre of young floral primordia from early stages on. MiR172 also accumulates in the shoot meristem upon floral induction, compatible with its known role in regulating AP2-related genes with a role in flowering. Furthermore, it showed that AP2 can cause striking organ proliferation defects that are not limited to the centre of the floral meristem, where its antagonist AG is required for terminating stem cell proliferation. Moreover, AP2 never expands uniformly into the centre of ag mutant flowers, while miR172 is largely unaffected by loss of AG activity. A model presented in which the decision whether stamens or petals develop is based on the balance between AP2 and AG activities, rather than the two being mutually exclusive (Wollmann *et al.*, 2010).

miRNA and shoot and root development

Cup-shaped cotyledon 1 (CUC1) and CUC2 are two important transcription factors of the NAM/ATAF/CUC (NAC)-domain transcription factor family, which is restricted to plants. They play important roles in both embryo-genic and floral development. Loss-of-function of these genes resulted in abnormal floral and shoot development.

Recently, five members (CUC1, CUC2, NAM, NAC1, At5g07680, and At5g61430) of the NAC domain gene family in *Arabidopsis* were identified that have complementary sites with miR164 and are targets of miR164. Of these, NAC1 is involved in lateral root development, whereas CUC1 and CUC2 regulate meristem development and separation of aerial organs. Over expression of miR164 resulted in the fusion of vegetative and floral organs, unbalanced floral organ numbers, and reduced lateral root emergence. In contrast, miR164-resistant mutants of CUC1 and CUC2 resulted in *Arabidopsis* embryonic, vegetative, and floral development defects, including misshaping of rosette leaves, altering flower structure, and changing cotyledon orientation. miR164 also controlled organ boundaries and root formation by regulating NAC1 and

CUC2 expression. Loss-of-function miR164 mutants accumulated NAC mRNA, resulting in more lateral root formation. In contrast, over expression of miR164 in wild-type *Arabidopsis* resulted in decreasing NAC1 mRNA levels and decreased lateral root emergence. miR164 plays an important role in controlling shoot and root development by regulating several NAC transcription factors. It may be possible to increase crop yields and resistance to environmental stress by appropriately controlling miR164 expression (Gau *et al.,* 2005 and Raman *et al.,* 2008).

miRNA and vascular development

HD-ZIP proteins also regulate vascular development as well as lateral organ polarity and meristem formation. ATHB15, a member of the HD-ZIP family, is predominantly expressed in vascular tissues, suggesting that it may play some role in plant vascular development. ATHB15 is one target of miR166. 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. This regulation mechanism may exist in all vascular plant species. In addition, some miRNAs may function in the biosynthesis of cell wall metabolites or cotton fiber development (Kim *et al.,* 2005).

miRNAs and involved in plant disease

Pathogen infection is an important biological factor that extensively affects plant growth and development. Pathogen infection causes about a 30 per cent yield loss for a majority of crops and fruits. In the long-term, plants have evolved complicated mechanisms to resist infection.

One of these mechanisms is pathogen-induced post-transcriptional gene silencing (PTGS). Recently, more and more evidences have showed that miRNAs are involved in plant diseases caused by different pathogens; some of them may also be involved in virus-induced gene silencing. Helper component-proteinase (HC-Pro), p19, p21, and p69, are unrelated viral suppressors of gene silencing. They play important roles in the virus response to plant antiviral silencing response. These suppressors are usually called pathogenicity factors, and they cause disease and various developmental abnormalities. Recently, several studies have showed that these suppressors are involved in the regulation of several miRNA activities. HC-Pro decreased miRNA levels, interfered with miR171 activity, and caused miR171- related developmental defects. When expressing HC-Pro gene in plants, 8 of 10 miR171-guided cleavage target mRNAs accumulated to elevated levels, and caused TuMV- and other virus-induced diseases. One possible mechanism is that HC-Pro, p19, and p21 inhibited the formation and/or activity of miRNA-containing RISC complex and resulted in failure of PTGS in infected plants. In contrast, another viral suppressor, p69, encoded by turnip yellow mosaic virus (TYMV), increased the transcription levels of a Dicer mRNA and miRNAs, and enhanced the miRNA-guided cleavage of host mRNAs. This suggested that p69 may enhance miRNA-mediated gene silencing and consequently enhance plant resistance to pathogens. Further clarification of this may provide tools for improving crop resistance (Kasschau *et al.,* 2003).

Future thrust

MicroRNAs down regulated the gene expression by cleaving mRNA or by repressing mRNA translation. As such, it may be possible to design artificial miRNAs to suppress target gene expression in order to study gene function, similar to the use of antisense mRNA and RNAi which are widely used as tools for studying gene function. Another possibility is the use of miRNA knowledge to improve plant yields, quality, or resistance to various environmental stresses including insect and pathogen infection. For example, crop resistance to drought could be improved through the induction of more lateral roots by down regulating miR164. Further study of miRNAs could provide us with new tools for increasing crop yield and/or improving crop quality.

CONCLUSION

Plant development is a tightly controlled process that is regulated at various levels of gene expression. Plant miRNA regulated the expression of many transcription factors that control aspect of development ranging from the development of various organs, their identity, patterning, polarity and developmental timing, to specific tissue or cellular organelle development. Various plant hormone signalling pathways intersect with set of transcriptional factors frequently via plant miRNA to ensure a proper development of plant.

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Table 1 : MicroRNA gene families conserved in plants.

miRNA Family	***Arabiopsis***	***Oryza***	***Populus***
miR 156	12	12	11
miR 159/319	6	8	15
miR 160	3	6	8
miR 162	2	2	3
miR 164	3	5	6
miR 166	9	12	17
miR 167	4	9	8
miR 168	2	2	2
miR 169	14	17	32
miR 171	4	7	10
miR 172	5	3	9
miR 390	3	1	4
miR 393	2	2	4
miR 394	2	1	2
miR 395	6	19	10
miR 396	2	5	7
miR 397	2	2	3
miR 398	3	2	3
miR 399	6	11	12
miR 408	1	1	1
miR 403	1	0	2
miR 437	0	1	0
miR 444	0	1	0
miR 445	0	9	0
Total	92	127	169

Source: Jones-Rhoades et al. (2006)

Table 2: Consequences of miRNA over expression

Sr. No.	miRNA	Target family	Consequences of over expression
1	miR156	SPL transcription factors	Increased leaf initiation, decreased apical dominance, delayed flowering time
2	miR159	MYB transcription factors	Male sterility, delayed flowering time
3	miR319	TCP transcription factors	Uneven leaf shape and curvature, late flowering
4	miR160	ARF transcription factors	A gravitropic roots with disorganized root caps, increased lateral rooting
5	miR164	NAC domain transcription factors	Organ fusion, reduced lateral rooting
6	miR166	HD-ZIP transcription factors	Seedling arrest, fasciated apical meristems, female sterility
7	miR172	AP2-like transcription factors	Early flowering, lack of petals, transformation of sepals to carpels
8	miR399	Ubiquitin-conjugating enzyme	Phosphate accumulation

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