

Overview of MicroRNA Biogenesis, Mechanism, and their Role in Crop Improvement

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ABSTRACT

Crop improvement mainly relied upon either existing variation or by creating new variation by altering DNA sequence. However, gene expression changes are also being considered to be important for improvement of various traits in crops. MicroRNAs are one such regulators of gene expression at post transcriptional level. MicroRNAs are small 20-24 nucleotide RNA molecules that cleave corresponding mRNA or inhibit its translation. In view of this, we will discuss biogenesis of miRNAs, techniques to determine their function, their role in controlling various traits, strategies to use miRNAs for crop improvement, challenges associated with miRNA based crop improvement and prospects for using miRNAs in crop improvement.

INTRODUCTION

In the current scenario of rapid population increase and global climate changes there is a need to increase crop production to significant levels. Breeders generally utilize existing variability or try to generate new

variability by altering the DNA sequence and exercise selection on such variation. However, other aspect whereby, gene expression can be modulated holds significant importance in crop improvement. RNA interference (RNAi)

approach using microRNAs (miRNAs) is gaining popularity is effective in regulating gene expression. miRNAs are 20-24 nucleotide long single strand non-coding RNA molecules that bind to target mRNA to modulate its expression. They are transcribed by RNA polymerase II from *MIR* genes and further processed by various enzymes to finally produce 20-24 nucleotide long miRNAs that regulate gene expression. They are involved in plenty of developmental processes in organisms and control many agronomic traits in crop plants.

Biogenesis of miRNA and molecular mechanism involved in gene regulation

Akin to coding genes, miRNAs are transcribed from miRNA/*MIR* genes by RNA polymerase II into long primary transcripts called as primary/pri miRNA (**Fig. 1**). Thereafter, pri-miRNAs are cleaved by RNaseIII-like enzymes called DICER-LIKE (DCL 1) in conjunction with other proteins into precursor/pre miRNAs. These pre-miRNAs are further processed by DCL1 into 20-24 nucleotide long miRNA:miRNA duplexes. Duplexes are then methylated at 3' end by *HUA enhancer 1* and exported to cytoplasm by EXPORTIN-5. Duplexes are then loaded into RNA-induced silencing complex (RISC) that contains ARGONAUTE (AGO) proteins. From miRNA: miRNA duplexes only one of the RNA strands is loaded onto RISC whereby, another strand is degraded by small-RNA degrading nuclease. Finally, loaded miRNA targets the RISC to its complementary mRNAs whereby, it may lead to two outcomes depending upon level of its complementarity with target mRNA. If miRNA is highly homologous to target mRNA it may lead to site-specific cleavage of mRNA whereas weak base pairing with target mRNA leads to translational repression (**Fig. 1**).

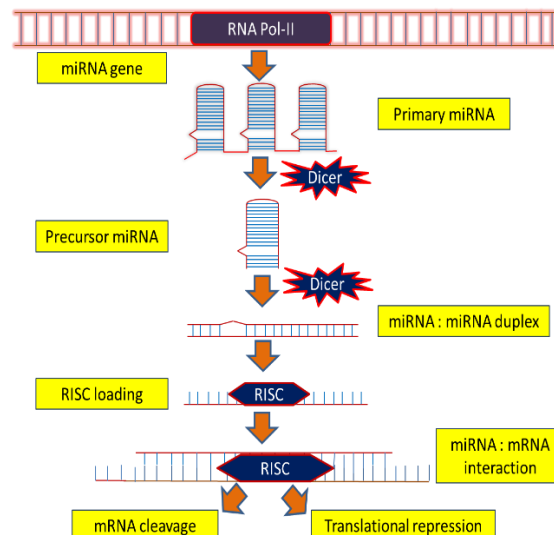


Fig. 1: Steps involved in biogenesis of miRNAs and gene regulation by miRNAs

Techniques to determine miRNA functions in plants

There are two most popular approaches to determine functions of various miRNA genes *viz.* gain of function analysis and loss of function analysis techniques. In case of gain of function approach, both pre-miRNA and full-length cDNA of *MIR* genes are overexpressed in a host using a strong constitutive 35S promoter and resultant phenotypic effects are observed in plants. In loss of function approach, *MIR* genes are rendered non-functional by targeting these genes through various gene-editing tools. Any corresponding phenotypic changes are considered as primary evidence of *MIR* gene function although, further confirmation is required. Instead of targeting *MIR* genes, their target genes can also be manipulated. In this approach, the cleavage-resistant target genes are overexpressed in host plant (Li and Millar, 2013). Cleavage-resistant forms of target genes are generated by changing miRNA cleavage target sites using synonyms mutants, as it results in translation into same amino acids. Resultant change in phenotype can be associated with corresponding *MIR* gene. In addition, one of the most common place methods of miRNA functional analysis is to

isolate, clone and sequence small RNAs and using sequence similarity search tools such as basic local alignment search tool (BLAST) to find their complementarity with genic sequences in the genome of corresponding crop.

Roles of miRNAs in governing various traits

Due to their role in regulation of myriad of genes, miRNAs are involved in growth and development, signal transduction, maintenance of genome integrity, innate immunity and response to different abiotic and biotic stresses. Functional roles of miRNAs along with their targets have been validated in various important crops. *osamiR156* is involved in regulating expression of *OsSPL13*, *OsSPL14*, *OsSPL16* genes in rice that are involved in many yield related traits (Jiao *et al.*, 2010). In wheat, *tae-miR164* targets *TaNAC21/22* that leads to immune response in plants (Feng *et al.*, 2014). Apart from these, there are innumerable studies indicating roles of miRNAs in various important agronomic traits such as floral development, drought tolerance, nutrient ion uptake, cold tolerance, photoperiod-sensitive male sterility, heading time, plant architecture and many more.

miRNA based strategies to improve agronomic traits in crop plants

Natural variation is a primary source for improvement in any crop, so looking into any natural variation at miRNA and miRNA target positions can be most rewarding. In fact, small nucleotide variations both at miRNA and miRNA target regions were found to be correlated with agronomic traits indicating possibility to utilize such variation in crop improvement (Zhao *et al.*, 2015). Besides natural variation, most common method for crop improvement using miRNAs involves transgenic breeding whereby, either miRNA or target gene is over expressed in modified form. These methods include constitutive

overexpression of miRNAs or targets, expression of miRNA resistant targets, artificial miRNAs and artificial target mimics. Wherein, miRNA resistant targets are modified versions of genes that do not contain miRNA target region due to use of synonymous mutations in the modified gene; artificial miRNAs are synthetically designed miRNAs to target specific genes of interest; artificial mimics are small RNA molecules that contain miRNA target sites with small mismatch or bulge that leads to entrapment and consequent decay of the miRNA molecule. Artificial miRNAs can also be used to target any gene to modulate its expression resulting in desired traits. More recent gene-editing techniques such as CRISPR-Cas system can be effectively used to edit *MIR* genes or miRNA target regions present in genes.

Challenges in miRNA-based strategies for crop improvement

Although miRNA technology looks very rewarding at first, still there are some challenges associated with the use of this technology in crop improvement. First of all, many traits including yield are controlled by several genes and tuning expression of all such genes at specific developmental stages is quite challenging. Secondly, some miRNAs may modulate gene expression only under specific environmental conditions and certain cell types. Additionally, as miRNAs regulate expression of multiple genes that in turn affect many traits, the unintended pleiotropic effects should be evaluated thoroughly before their wide scale implementation in crop improvement. Apart from that, CRISPR-Cas system also faces some challenges in targeting *MIR* genes especially when they reside in intronic regions, as it gives limited space for targeting *MIR* genes without affecting neighboring genes. Finally, due to public safety, transgenic crops face some regulatory challenges, so crops improved using miRNA

based strategy involving transgenic techniques could face some hurdles.

CONCLUSION AND PERSPECTIVES

Hitherto, tremendous progress has been made in understanding miRNA biogenesis and their effect on gene regulation. It got abundantly clear that miRNAs play crucial roles in various biological processes in organisms. miRNAs seem to be controlling almost all important agronomic traits starting from various kinds of stresses to yield related traits in crop plants. Suitable strategies have been developed to exploit miRNAs to improve agronomic traits. Many such strategies either try to exploit natural variation in miRNA or miRNA target regions or they use transgenic breeding to control expression of target genes. Although, such strategies are deemed rewarding, they often come with some challenges, especially transgenic breeding, where, policy restrictions exist. Gene editing techniques such as CRISPR/Cas system is also facing some challenges in miRNA related editing. In view of all these opportunities and challenges it is clear that miRNAs have huge potential to improve several traits in crop plants. Further discovery and validation of more miRNAs as well as development of additional miRNA-based strategies can give fruitful outcomes in the field of miRNA based crop improvement.

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