# *Micro-RNAs in Crop Improvement: Fine tuners for Complex Traits*

**Sainath K[1]*, Praveen K[3], B P Maruthiprasad[4], Akshay M[2], Eshwar[1] and Mahalakshmi A[1]**

[1]*Department of Genetics and Plant Breeding, UAS, Raichur*
[2]*Department of Genetics and Plant Breeding, UAS, Dharwad*
[3]*Department of Agricultural Microbiology, UAS, Bangalore*
[4]*Department of Genetics and Plant Breeding, UAS, Bangalore*

Corresponding Author
Sainath K
Email: sainathkarbhari98@gmail.com

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## ABSTRACT

To sustain global food security under the growing pressures of population expansion, economic development, and climate change, agricultural productivity must be enhanced through sustainable and innovative approaches. Genetic crop improvement integrating advances in molecular biology, biotechnology, genomics and plant physiology offers a powerful means to achieve this goal. However, the development of superior crop varieties often encounters the challenge of trait trade-offs, where the expression of one desirable characteristic can compromise another. In this context, microRNAs (miRNAs), a class of small non-coding RNAs that regulate gene expression at the post-transcriptional level, have emerged as precise and versatile molecular tools. Since their discovery in *Caenorhabditis elegans* in 1993, miRNAs have been recognized as master regulators influencing plant development, signal transduction, stress tolerance and disease resistance. Their ability to fine-tune gene expression without permanently altering genomic sequences makes them valuable targets for molecular breeding strategies aimed at achieving high yield, resilience and sustainability in crops.

## INTRODUCTION

To meet the growing challenges stemming from rapid population growth, economic development and global climate change, crop production should be significantly increased in a sustainable manner in the future. Genetic crop improvement or molecular breeding, which integrates advances in plant physiology, genetics, biotechnology and genomic research, can contribute towards achieving this goal. One of the critical requirements for genetic crop improvement is the introduction of new genetic material into crop lines of interest, which can be accomplished by introducing single or multiple genes via genetic engineering or marker assisted selection

One of the most common challenges for both conventional and modern crop improvement is that the appearance of one desirable trait in a new crop variety is always balanced by the impairment of one or more other beneficial characteristics. The best way to overcome this problem is the flexible utilization of regulatory genes, especially genes that provide more efficient and precise regulation in a targeted manner. MicroRNAs (miRNAs), a type of short non-coding RNA, are promising candidates in this area due to their role as master modulators of gene expression at the post-transcriptional level, targeting messenger RNAs for cleavage or directing translational inhibition in eukaryotes (Tang *et al.,* 2017).

### Types of RNA

1. **Coding RNA:** RNA serves as a template for protein synthesis.

- **mRNA (Messenger RNA:** It is a product of transcription. It is a linear single stranded polynucleotide chain. It forms about 3 to 5% of the total RNA content. It consists of about 900 to 1500 nucleotides. It has no base pairing (Attardi. G *et al.,* 1963).

✓ It carries the genetic information from DNA to proteins in the form of codons.

2. **Non-coding :** RNA has several various regulatory structural roles in the cell without being translated into proteins (Santosh *et al.,* 2015).

✓ **rRNA (Ribosomal RNA) :** It is most abundantly (largely) occurring RNA in the cell. it forms about 80% of the total RNA content. It forms major component of ribosome. it has many folded regions, folded regions have base pairing i e, A=U & C≡ G. It consists of 120 to 4500 ribonucleotides. It is also known as Structural RNA.

- It helps to form the structure of ribosome and helps to bind the mRNA and tRNA to ribosome and rRNA directs the translation of mRNA into proteins.

✓ **tRNA (Transfer RNA) :** It forms about 3 to 15% of the total RNA content. It is shortest RNA consists of about 80 ribonucleotides. tRNA folded itself to produce double stranded regions. The folded regions have base pairing i.e., A=U and C= G. It is also known as Soluble RNA (S RNA) or Adaptor RNA

- tRNA transfer amino acid to ribosome (site of protein synthesis) during protein synthesis. Transfer RNA brings or transfers amino acids to the ribosome that corresponds to each three nucleotide codon of rRNA. The amino acids then can be joined together and processed to make polypeptides and proteins.

✓ **snRNA (Small nuclear RNA) :** These are a class of non-coding RNA molecules found in the nucleus of eukaryotic cells, primarily involved in RNA processing and splicing. They are a crucial component of the spliceosome, a large complex that removes introns from pre-mRNA.

- ✓ **siRNA (Short interfere RNA) :** small interfering RNA, is a double-stranded RNA molecule, typically 20-25 nucleotides long, that plays a crucial role in gene silencing through a process called RNA interference (RNAi). It essentially inhibits the expression of a specific gene by binding to and degrading its corresponding messenger RNA (mRNA).
- ✓ **miRNA (Micro RNA) :** A microRNA is a small non-coding RNA molecule found in plants, animals and some viruses

### Discovery and Background

The evolution of complex multicellular organisms from single-celled ancestors necessitated more sophisticated gene regulation systems. While transcriptional regulation via DNA-binding proteins was already known, another layer—**post-transcriptional regulation**—remained undiscovered until the early 1990s.

In 1993, **Victor Ambros** and **Gary Ruvkun** made a groundbreaking discovery while studying developmental mutants of the nematode *Caenorhabditis elegans* (*C. elegans*). They were particularly focused on genes called **lin-4** and **lin-14**,which controlled developmental timing (heterochrony) (Dusan, B., 2024)

### The Discovery of lin-4 and its Unusual Nature

Ambros's lab cloned *lin-4* and made a surprising finding—it did not encode a protein but rather a small 22-nucleotide RNA. At the same time, Ruvkun's lab showed that *lin-14* protein expression was regulated via its 3' untranslated region (3'UTR) by partial sequence complementarity with the lin-4 RNA. This was the first evidence of **a new class of small non-coding RNAs** that regulate genes at the RNA level—now known as **microRNAs (miRNAs)**.

Their independent discoveries were published back-to-back in *Cell* (1993) and introduced the concept of **post-transcriptional gene regulation** via RNA-RNA interaction (Drula, R. and Calin, G.A., 2025).

### Explosion of miRNA Research

The discovery of let-7 sparked a surge in miRNA research. Labs led by **Ambros**, **Bartel**, and **Tuschl** began cloning and identifying miRNAs from various organisms. Thousands of miRNAs were found, including in viruses. With advances in sequencing and bioinformatics, **miRBase** (a miRNA database) now contains over 48,000 mature miRNA sequences from 271 organisms (Niazi, S.K. and Magoola, M., 2024).

### ORIGIN of miRNA

- Endogenous origin
- Generated in Nucleus
- Generated by RNA polymerase II or III after post transcriptional modification
- Processes mainly from introns and very less from exons
- Regulated by own promoters
- Sometimes transcribed as long transcript

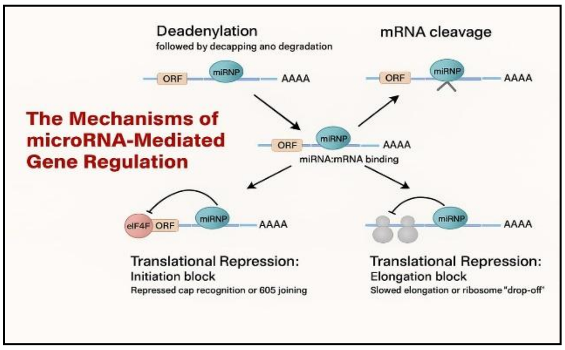
### The mechanisms of microRNA mediated gene regulation (Rani, V. and Sengar, R.S., 2022)

- ❖ **mRNA cleavage :** When particular miRNA is perfectly complementary to mRNA then the target mRNA is degraded means that is cleaved by Argounate proteins which contains domain for RNAaseH.

- **Initiation block** : When miRNA has partial complementary sequence with that mRNA , it binds and blocks initiation. This happens because during translation polyA needs to interact with 51 initiation protein. So this miRNA inhibit polyA activity and blocks initiation process.

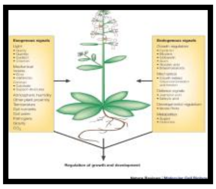
- **Elongation block** : When it binds in imperfect manner, it will not allow ribosomes to go ahead results in slow elongation.

- **Deadenylation** : There is a complex called CCR4 which is responsible for removing adenine from mRNA followed by decapping and degradat



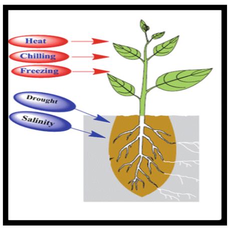
## FUNCTIONS OF miRNA IN PLANTS

- **Regulate plant development** : miRNAs are involved in regulating diverse developmental processes including root growth & development, shoot apical meristem development, leaf morphogenesis and floral organ formation.



- **Signal transduction** : They acts as key regulators influencing the responsiveness of cells to extracellular signals and shaping cellular responses.

- **Plant disease resistance** : miRNA contribute to plant defence mechanism against pathogens by regulating the expression of genes involved in defence pathway and hormone signaling.



- **Environmental stress response** : miRNA helps plants adopt to various environmental stresses like drought, salinity and pathogen attack by regulating the expression of stress related genes.

## CONCLUSION

MicroRNAs (miRNAs) represent a fundamental layer of gene regulation that adds complexity and flexibility to plant genetic networks. Their discovery has revolutionized our understanding of how plants control growth, development and adaptation to environmental stress. By regulating key transcription factors and stress-responsive genes, miRNAs play crucial roles in balancing physiological and developmental processes. Harnessing miRNA-based regulatory pathways provides new opportunities for improving crop performance through molecular breeding and biotechnological interventions. Future research focusing on miRNA identification, functional characterization and precise manipulation will be vital for developing climate-resilient, high-yielding and sustainable crop varieties to meet the global challenges of agriculture in the 21st century.

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