# The Emergence of RNA Interference Technology: An Experimental & Innovative Approach for Crop Improvement

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**ABSTRACT**

Plant breeders use various biotechnological tools to change the expression of genes. For the expression of any gene, specific gene regulation mechanisms are required. RNA interference is a highly conserved gene regulatory mechanism that controls the expression of genes at post transcriptional level. It involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA. It has tremendous potential to improve the crop plants against different types of biotic and abiotic stresses, nutritional enhancement and for better quality traits. Ultimately, this technology plays a vital role to counter global food security and also to maintain sustainability.

## INTRODUCTION

Crop improvement is the manipulation of genotypes such that it will be of maximum usefulness to humans. It has been estimated that global food production must increase by 70% by 2050 to meet the demand due to the growing global population. Advances in molecular biology, especially biotechnology, offer several advantages over traditional approaches of plant breeding. Among different biotechnological tools, RNA Interference has been playing important role in crop improvement. RNA interference (RNAi) is a term coined by Fire and his team in 1998 (Fire *et al*. 1998) when they discovered that

injection of dsRNA into the nematode worm *Caenorhabditis elegans* resulted in potent and specific silencing of expression of the endogenous gene homologous to the RNA. RNAi can be defined as the ability of exogenous or endogenous double stranded RNA to suppress the expression of the gene which corresponds to the sequence of double stranded RNA (Dash *et al*. 2015).

**What is RNAi?**

RNA interference is a process which involves silencing of a specific mRNA due to a complementary dsRNA molecule that binds to and prevents translation of the mRNA (silencing). The source of this dsRNA could be from an infection by viruses having RNA genomes or mobile genetic elements (transposons). This dsRNA can be introduced through injection, spray or using Agrobacterium vectors into the host organisms. It has been widely used as a knockdown technology to analyse gene function in various organisms. It is known as 'quelling' in case of fungi, 'PTGS' in plants and 'RNAi' in animals. It is found in all eukaryotes, *viz.*, Kingdom protista, fungi, plantae and animalia but it is absent in prokaryotes (Kingdom Monera).

RNAi is a novel approach to modify the gene expression for better quality traits and nutritional improvement in different crops. It has opened new avenues for the development of eco-friendly techniques for crop improvement as specific genes are suppressed which cause stress and expression of novel genes for disease resistance (Younis *et al*. 2014). With advancement in technology, RNAi can help in the development of improved crops with higher productivity and better quality to meet the demand of a growing population.

**History of RNAi:** Molecular biologists had been working from a long time to knockout gene expression at the mRNA level. Guo antisense experiment, using either DNA or RNA are relatively simple techniques for probing gene functions; however, these methodologies have suffered drawbacks due to lack of specificity and incomplete efficiency. However, the desired effects were difficult to predict and often only a weak suppression was achieved.

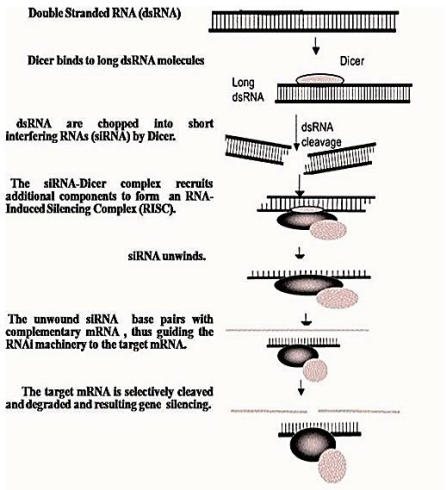
Post-transcriptional gene silencing (PTGS) in plants, quelling in *Neurospora* and RNAi in animals have been discovered independently but share a common conserved mechanism. This kind of process is also found in protozoa like Paramecium and trypanosomes. In both PTGS and RNAi, the presence of dsRNA triggers the Dicer or RISC system for RNA degradation. The strand of the dsRNA which is identical in sequence to a region in target mRNA molecule is called the sense strand and the other strand which is complimentary to the target mRNA is termed as antisense strand. An enzyme complex called Dicer in *D. melanogaster* which is thought to be similar to RNase III, then recognizes dsRNA and cuts it into roughly 22-nucleotide long fragments. These fragments are termed as small interfering RNA (siRNA) act as templates for the RNAi inducing silencing complex and hence, specifically suppresses its expression. PTGS was actually seen in plants several years before RNAi was discovered in animals. PTGS was detected when extra copies of plant genes were inserted into the plant cells by genetic engineering. Instead of increased levels of gene expression, as thought, sometimes the result was a massive reduction in gene expression. The probable reason is the destruction of mRNA corresponding to the sequences which are inserted and occurs by a mechanism closely related to RNAi.

The milestone discovery in this field however came from the experiment of Fire and Mello when they injected both sense and antisense strands simultaneously into the nematode

worms and saw interference. The mixture of sense and antisense strands silenced a targeted genes expression ten times more efficiently than either strand alone. Fire and Mello saw this effect that was induced by dsRNA as a novel one and called the process RNA interference (Fire *et al*. 1998).

**Mechanism of RNAi:** RNA interference involves small RNA molecules called short interfering RNA (siRNA) or microRNA (miRNA). These molecules are 21 to 28 base pair long and produced from larger, dsRNA molecules by the enzymatic action of Dicer which are dsRNA-specific endonucleases. The nematode worm *Caenorhabditis elegans* produces a single kind of Dicer enzyme whereas *Drosophila* produces two and *Arabidopsis* produces at three different Dicer enzymes. In *C. elegans* and *Drosophila*, Dicer enzyme acts in the cytoplasm and in *Arabidopsis*, it acts in the nucleus.

The siRNA and miRNA produced by Dicer enzyme are base paired throughout their lengths except at their 3' ends, where two nucleotides are unpaired. In the cytoplasm, siRNA and miRNA become incorporated into ribonucleoprotein. The ds-siRNA or miRNA in ribonucleoprotein is unwound and one of its strands is removed. The remaining single strand of RNA then interacts with specific mRNA molecules. This interaction is catalysed by base pairing between the single strand of RNA in the ribonucleoprotein complex and a complementary sequence in the mRNA molecule. Because this interaction prevents the expression of the gene that produced the mRNA, the ribonucleoprotein complex is known as RNA Induced Silencing Complex (RISC). RISCs from different organisms vary in size and composition. However, they all contain at least one molecule of Argonaute (AGO) protein. Whenever the base pairing between the RNA within the RISC complex and the target sequence in the mRNA is perfect, an AGO protein in the RISC behave like an endonuclease to cleave the target mRNA in the middle of the base paired region and hence, performs the "slicer" function. The cleaved mRNA is then degraded. After cleavage, the RISC may associate with another molecule of mRNA and induce its cleavage. Because a RISC may be used consequently without losing its ability to target and cleave mRNA, it acts as a catalyst. RISC-associated RNA that result in mRNA cleavage are usually termed short interfering RNA. Whenever the RNA within the RISC pairs imperfectly with its target sequence, the mRNA is usually not cleaved and results in the inhibition of translation of the mRNA. RISC-associated RNA that have this kind of effect are commonly regarded as microRNA. In animals, the sequences targeted by RISCs are found in the 3' untranslated regions of mRNA molecules whereas in case of plants, the sequences targeted by RISCs are located within the coding region of the mRNA, or within the 5' untranslated regions of mRNA.




| | miRNA | siRNA |
|---|---|---|
| **Origin** | **Endogenous** | **Exogenous** |
| **Processing** | **Drosha & Dicer** | **Dicer** |
| **Target specificity** | **Imperfect base pairing** | **Perfect base pairing** |
| **Primary function** | **Inhibition of translation** | **Degradation of mRNA** |

***Figure: Mechanism of RNAi***

**Amplification and the spread of RNAi:** Almost less than 50 molecules of siRNA have the ability to silence target RNA that is present in thousands of copies per cell in an organism which is possible due to amplification of the siRNA via an RNA-dependent RNA polymerase (RdRP) which doesn't need a primer. The cutting of the target mRNA by Slicer produces two aberrant and unstable RNA molecules, one is capped but without the poly(A) tail and the other one is with a tail but no cap. One or both of these aberrant RNA molecules are apparently used as template by RdRP to generate dsRNA. The dsRNA, then acts as a substrate for Dicer to produce more siRNA and hence, amplify the RNA interference effect.

Further, RNAi effect is also capable of spreading from cell to cell and may travel considerable distances through an organism. This effect is particularly noticed in case of plants. Spreading of the siRNA signal throughout the body is also seen in animals. Remarkably, in *C. elegans* the RNAi effect is passed on for several generations (without alterations in the genomic DNA sequence of the targeted gene occurring). But mammals don't possess the RdRP responsible for RNAi amplification and hence, RNAi remains relatively localized.

**Application of RNAi in crop improvement:**

**Wheat**: RNAi has been used to down regulate the two different isoforms of starch-branching enzyme (SBE) II (SBEIIa and SBEIIb) in wheat endosperm to increase its amylose content (>70%) to improve public health (Tang *et al*. 2007).

**Maize**: RNAi has been successfully used to produce a high lysine maize by suppressing the expression of the 22-kD maize zein storage protein which is poor in lysine content and hence, produced quality and normal maize seeds with high levels of lysine-rich proteins (Houmard *et al*. 2007).

**Rice**: Kusaba and his co-workers developed a rice variety called LGC-1 (low glutenin content 1) which is quite helpful to the kidney patients which are unable to digest glutenin (Kusaba *et al*. 2003).

**Barley**: Bayer Crop Science has developed barley varieties which are resistant to viral infection, *viz.*, BYDV (Barley yellow dwarf virus) (Wang *et al*. 2000).

**Banana**: RNAi has been used to combat Fusarium wilt in banana (Jayasekara *et al*. 2025) and Banana Bract Mosaic Virus (BBrMV), which leads to decline in the banana production in South East Asia and India (Rodoni and Dale 1999). RNAi vector was aimed at silencing the Coat Protein (CP) region of the virus to develop banana that is resistant to BBrMV.

**Cotton**: RNAi is used to develop cotton varieties with increased drought tolerance during germination and development under normal and drought conditions (Qin *et al*. 2022). A RNAi construct of the *d-cadinene synthase* gene of gossypol synthesis fused to a seed-specific promoter resulted in reduction of gossypol content in seed as well as to produce seeds with higher nutritional value (Kumar *et al*. 2006).

**Soybean:** RNAi used to down regulate omega-3 fatty acid desaturase (*GmFAD3A*, *GmFAD3B* and *GmFAD3C*) which resulted in lowering the alpha-linoleic acid content (Flores *et al*. 2008).

**Jute**: RNAi used to down regulate a key enzyme (4-coumarate: CoA ligase) responsible for biosynthesis of lignin in jute.

**Tomato**: RNAi used to down regulate *DE-ETHIOLATED1* gene (*DET1*) under fruit-specific promoters to enhance carotenoid and flavonoid levels in tomato fruits with lower effects on plant growth and other fruit quality parameters (Davuluri *et al*. 2005).

RNAi technology is also used to increase shelf life in tomato by suppressing the expression of *ACC oxidase* gene in tomato (Xiong *et al*. 2005).

**Coffee**: RNAi used for the creation of Decaffeinate coffee (DECAF) variety that produces natural coffee with lower caffeine content (Van Uyen 2006).

Besides, RNAi is also used for development of male sterility and fertility like in case of tobacco and tomato where RNAi was used to suppress the expression of *Msh1* gene which led to rearrangements in the mitochondrial DNA associated with naturally occurring cytoplasmic male sterility (Sandhu *et al*. 2007).

## CONCLUSION

RNA interference (RNAi) is an advance technology which is highly effective and powerful tool of functional genomics for silencing the gene expression for crop improvement. At Present, research is going on RNA interference in India at various institutions like National Institute of Plant Genome Research (NIPGR), New Delhi, Jamia Hamdard University, South Delhi, Indian Institute of Science Education and Research (IISER) Kolkata and Pune, ICAR-Indian Institute of Oilseeds Research (IIOR), Hyderabad, Indian Institute of Technology Guwahati (IIT Guwahati) and Division of Nematology, IARI, New Delhi to develop disease, insect and virus resistant, nutritionally rich and toxic free crops which will ultimately help to meet the growing needs of humans and sustaining agriculture.

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