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RNA interference (RNAi)- A promising technology for sustainable agriculture

RUPALI KOTHAWADE, B.A. AGLAVE* AND M.O. LOKHANDE
Department of Biotechnology, H.P.T. Arts and R.Y.K. Science College, NASHIK (M.S.) INDIA

Key words :

RNA silencing is a novel gene regulatory mechanism which regulates the transcript level by either suppressing transcription (transcriptional gene silencing [TGS]) or by activating a sequence-specific RNA degradation process (post- transcriptional gene silencing [PTGS] or RNA interference).

RNA interference (RNAi) is a conserved biological response to double-stranded RNA that medicates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids, and regulates the expression of protein coding genes. This natural mechanism for sequence-specific gene silencing promises to revolutionize experimental biology and may have important practical applications in functional genomics, therapeutic intervention, agriculture and other areas.

RNAi related events were described first in plants and later on in higher eukaryotes including protozoa, nematodes, insects, flies, parasites, mouse and human cell line. Three phenotypically different but mechanically similar forms of RNAi:

- Cosuppresion or PTGS in plants
- Quellinlg in fungi
- RNAi in animal kingdom.

Mechanism:

A class of small RNA that mediates the silencing of particular gene functions by interacting with mRNA often in 3'UTR, resulting in either mRNA degradation or translational inhibition mRNA and thus gene that produces it, is silenced. Small RNA are sometimes called micro-RNA (miRNA) and many small RNA which present only transiently during development are referred as small temporal RNA (stRNA). RNAs mainly act in 2 ways;

First, miRNA transcribed as precursor RNAs about 70 nucleotides long, with internally complementary sequences that form hairpin like structures. The precursor are cleaved by endonucleases in Dicer family (RNAse Ø) to form short duplexes about 20-25 nucleotides long. One strand of the processed miRNA is transferred to target mRNA, leading to inhibition of translation or degradation of RNA.

Second, double strand RNA can be constructed and introduce into a cell. Dicer processes the duplex RNAs into short segments called small interfering RNA (siRNA).these binds with target mRNA and silence it. This process is called as 'RNA interference'. In plants, virtually any gene can be effectively shut down in this way. In nematodes, simply introducing duplex RNA into worm's diet produces very effective suppression of the target gene.

PTGS in plants:

In plants, the RNA silencing story unfolded during a search for transgenic petunia flowers that were expected to be purple. In 1990 R. Jorgensen's laboratory wanted to up regulate the activity of gene for chalcone synthase (chsA), an enzyme involved in the production of anthocyanin pigments. Surprisingly, some of the transgenic petunia plants harboring the chsA coding region under the control of 35S promoter lost both endogene and transgene chalcone synthase activity, and thus many of the flowers were developed white sectors. The loss of cytosolic chsA mRNA was not associated with reduced transcription, as demonstrated by run-on transcription taste in isolated nuclei. Jorgensen coined the term cosuppression to describe the loss of mRNAse of both the endo and transgene.

Quelling and RNAi:

Homology dependent-dependent phenomenon was observed in fungal systems. These events were called 'quelling'. Quelling came to light during attempts to boost the production of an orange pigment made by the gene of fungus *Neurospora crassa*. An *Neurospora crassa* strain containing a wild-type all +gene (orange phenotype) was transformed with a plasmid containing a 1500bp fragment of the coding sequence of the wild type gene. A few transformants were stably quelled and showed albino phenotypes. In the all +quelled strains, the level of unspliced mRNA was similar to that of the wild-type strain, whereas the native all+ mRNA was highly reduced, indicating that quelling and not the rate of transcription affected the level of mature mRNA in a homology-dependant manner.