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Role of RNAi Induced Gene Silencing in Horticulture Crops

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Introduction

RNA interference (RNAi) is a method of blocking or silencing the gene function by inserting brief sequences of ribonucleic acid (RNA) that are partially match the sequence of the target gene, as a result no proteins are produced. In eukaryotic cells, RNA interference is a naturally occurring, evolutionarily conserved gene regulation event. It has developed to defend cells from invasive alien DNA. In addition to this, it supports epigenetic modification, genomic stability maintenance, transposon movement regulation, and transcriptional and translational control of cellular processes.

Discovery of RNAi technology

When Napoli *et al.* (1990) experimented to deepen the colour of petunia flowers by up regulating the gene coding for pigment production, which surprisingly resulted into variegated flowers instead of expected deep purple flowers, the gene silencing phenomenon was accidentally revealed in petunia flowers. The process was referred to as "co-suppression" because it resulted in the suppression of the expression of both a transgene and a homologous endogenous gene.

The same phenomenon in the nematode *Caenorhabditis elegans*, when they injected dsRNA in *C. elegans*, which resulted into effectively silenced the target endogenous gene that is homologous to RNA, hence the phenomenon was named RNA interference (RNAi). This turned out to be one of the most compelling discoveries in biotechnology, because of its targeted gene regulation, accuracy, and heritability. The Nobel Prize in Physiology or

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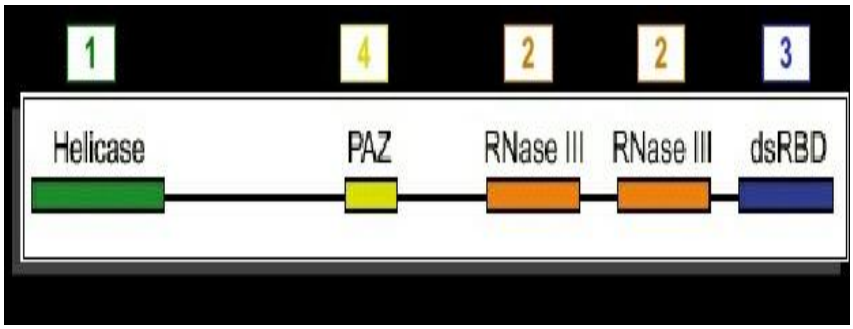
Medicine 2006 was awarded jointly to Andrew Z. Fire and Craig C. Mello "for their discovery of RNA interference - gene silencing by double-stranded RNA"

Salient features of RNAi

- Double stranded RNA rather than single stranded antisense RNA is the interfering agent.
- High degree of specific gene silencing with less effort.
- Highly potent and effective.
- Silencing can be introduced in different developmental stages.
- Systemic silencing.
- Silencing effects passed through generations.

Components of RNAi Mechanism

1. **Dicer:** Endoribonuclease (RNAse III family) cleaves the dsRNA into active small non-coding RNAs and initiates the RNAi pathway. It contains four domains namely, N-terminal helicase domain, a PAZ (Piwi/Argonaute/Zwille) domain, dual RNase III domains, and a dsRNA binding domain. These enzymes main function is to identify the dsRNA precursor from the RNAi pathway and produce short non-coding RNA of a particular length.



2. **RISC (RNA-induced silencing complex) :** RISC is a large (~500 kDa) RNA-multi-protein complex & Member of Agronaute family. RISC uses the siRNA or miRNA as a template for recognizing complementary mRNA. When it finds a complementary strand, it activates Argonaute (a protein within RISC) and cleaves mRNA.
3. **Argonaute:** Argonaute proteins are primarily found in bacteria, archaea, and eukaryotes. The Argonaute protein's key role is to identify and guide strand termini, use its nuclease ability to cut the target mRNA, or recruit other proteins involved in silencing.

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4. **RdRPs (RNA dependent RNA polymerase):** Plays key role in triggering and amplifying the silencing effect. dsRNAs formed are finally the targets for sequence specific mRNA degradation.
5. **Small Interfering RNA (siRNA):** Gene silencing through RNAi can be triggered via long dsRNA or short hairpin precursors, which can perfectly base pairs with the gene to be silenced. The introduction of long endogenous dsRNA directly into the cytoplasm or access of transgene, viral intruders, or transposable elements can ignite the RNAi pathway by recruiting the Dicer or Dicer-like enzymes.
6. **Microprocessor:** Another endonuclease in the RNAi pathway, known as Drosha which is a nuclear RNAse-III enzyme. Its domain structure consists of N terminus (rich in proline, arginine & serine), two RNA-III domain, one doublestranded RNA binding domain that cleave and processes pri-miRNA into pre-miRNA.

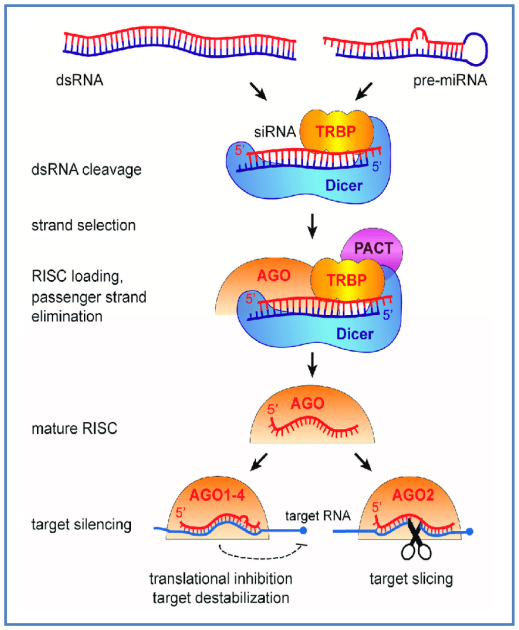

Fig 1: Mechanism of RNAi gene silencing technology

Applications of RNA interference (RNAi) in Horticulture Crops

1. **Modification of flower colour :** RNAi technology was used to establish genetically modified gentian plants with down regulated anthocyanin 5, 30 -aromatic acyltransferase (5/30 AT) and F3′ 5′ H functions, which are both required for gentiodelphin biosynthesis. From 15 transgenic gentian plants two lines of flower color modification were obtained (Nakatsuka *et al*., 2007)

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2. **Enhancement of nutritional quality:** In a white-fleshed sweet potato cultivar (cv. Yulmi), Kang *et al.* (2017) used the RNAi-*IbCHY*- construct to grow orange flesh, with total carotenoid and -carotene concentration in storage roots that were 2 and 16-fold higher than non-transgenic plants.
3. **Early ripening:** Early fruit ripening is a highly desirable and valuable trait. Fruit-specific suppression of the ethylene receptor *LeETR*4 causes early ripening in tomato.
4. **Increased shelf life:** Over expression of *mac-mir395* in transgenic bananas with RNAi integrated can delay ripening and lengthen shelf life. MaMADS-box genes (MaMADS-1, MaMADS-2) repressed banana fruits took 3–14 days longer to ripen after harvest.
5. **RNAi induced male sterility:** Transformed tomato lines expressed the siRNA in anthers showed the sterile and aborted pollen with defective morphology. These transgenic lines resulted in a considerable reduction in the transcript of SAMDC genes. And other polyamine synthesis genes, SPDSYN, and ADC transcript levels are also reduced in tomatoes, producing defective pollens (Sinha and Rajam, 2013).
6. **Biotic stress improvement:** PVY (Potato Virus Y) is a severe and common virus that affects a variety of major crops, notably potatoes. Myzus persicae vectors transmit this virus across plants in a non-persistent mode. By using RNAi assay reduce the 47 per cent of PVY transmission.

Conclusion

The RNAi technology has boosted our knowledge about gene regulation, gene function and gene analysis and opened up novel avenues to develop technologies with immense potential in plant protection and nutrition enrichment as well as many other areas related to crop improvement in horticulture crops. It is better than conventional transgenic technologies where they generally need expression of whole genes, where as RNAi requires comparatively small transgenes for silencing.

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