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RNA interference (RNAi): An Evolutionary Approach

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SUMMARY

Advances in science result in substantial advancements in scientific investigations, which eventually result to implications in human wellbeing. RNA interference (RNAi) is an evolutionarily conserved genetic regulation strategy that modulates expression levels at the post-transcriptional level and is engaged in sequence-specific epigenetics triggered by the insertion of dsRNA, resulting in transcriptional silencing or inhibition. Since the revelation of RNAi and its regulatory capabilities, it became obvious that RNAi has enormous promise for crop development. Antisense technology becomes less precise, efficient, and persistent than RNAi innovation.It has already been reliably used to modify plant gene expression for higher quality attributes. RNAi's impact on crop plants has revealed to be a promising approach in battling biotic and abiotic stressors, male sterility, delayed ripening, secondary metabolite alteration, and nutritional enrichment in terms of bio-fortification and bio-elimination.

INTRODUCTION

RNA interference (RNAi) is a scientific principle in which double-stranded RNA molecules restrict expression of genes in a sequence-specific manner via translational or transcriptional repression. Co-suppression, post-transcriptional gene silencing (PTGS), and quelling were all terms used in the history to describe RNAi. The existence of these apparently disparate mechanisms was discovered to be RNAi after a detailed investigation of each of them. Andrew Fire and Craig C. Mello shared the 2006 Nobel Prize in Physiology or Medicine for their work on RNAi in the nematode worm *Caenorhabditiselegans*, which they published in 1998.As specific genes that produce stress and expression of novel genes for disease resistance are inhibited, this strategy has opened up new possibilities in the development of environmentaly benign techniques for crop improvement and a method for reducing pests and diseases, introducing new plant characteristics, and increasing agricultural output. Researchers have used RNAi to create a variety of unique crops, including nicotine-free tobacco, non-allergenic peanuts, decaffeinated coffee, and nutrient-fortified maize etc. The RNAi process aids in the detection and functional assessment of multiple genes crucial for genetic improvement (Saurabh et al., 2014). RNA interference has recently emerged as a potent and dependable technology for suppressing the expression of gene products, as well as a tool for determining gene loss of function phenotypes, which refers to genetic linkage analysis. Biotechnologists believe that RNAi, a revolutionary tool in genetic engineering, has enormous potential to modulate expression levels of gene in plants for decent quality attributes and nutritional enhancement in several crops.

Mechanism and Principle of RNAi

RNA interference (RNAi) is a biological methodology that enables RNA molecules to restrict genes from being expressed. Short RNA molecules that seem to be complementary to endogenous mRNA are often designed and assembled into cells, where they adhere to the target mRNA. RNA interference (RNAi) refers to a mechanism in which minute fragments of RNA adhere to messenger RNAs that encode proteins and prevent them from being translated. RNA interference is a natural mechanism that plays a function in protein synthesis regulation and resistance.

RNAi occurs in four basic steps:

- Processing of long dsRNA by RNase III Dicer into siRNA duplexes,
- Loading of one of the siRNA strands on an Argonaute protein possessing endonucleolytic activity
- Target recognition through siRNAbasepairing, and
- Cleavage of the target by the Argonaute'sendonucleolytic activity.

How RNAi works

- The RNAi process of cells is activated when a prolonged dsRNA, such as a transgene, a rogue genetic element, or a viral incursion, enters the cell. This causes the enzymatic Dicer to be recruited.
- Dicer breaks down dsRNA into little RNAi pieces that are 20-25 bplong (siRNA).
- After that, an RNA-induced silencing complex (RISC) determines whether the two siRNA strands are sense or antisense. The targeted gene's sense strands are eliminated.
- The antisense strands are integrated into the RISC. These are employed as a sequence-specific guide to target messenger RNAs (mRNA).
- RISCcleaves mRNA which encode for amino acids. The activated RISC can participate in mRNA degradationrepeatedly, preventing protein expression.

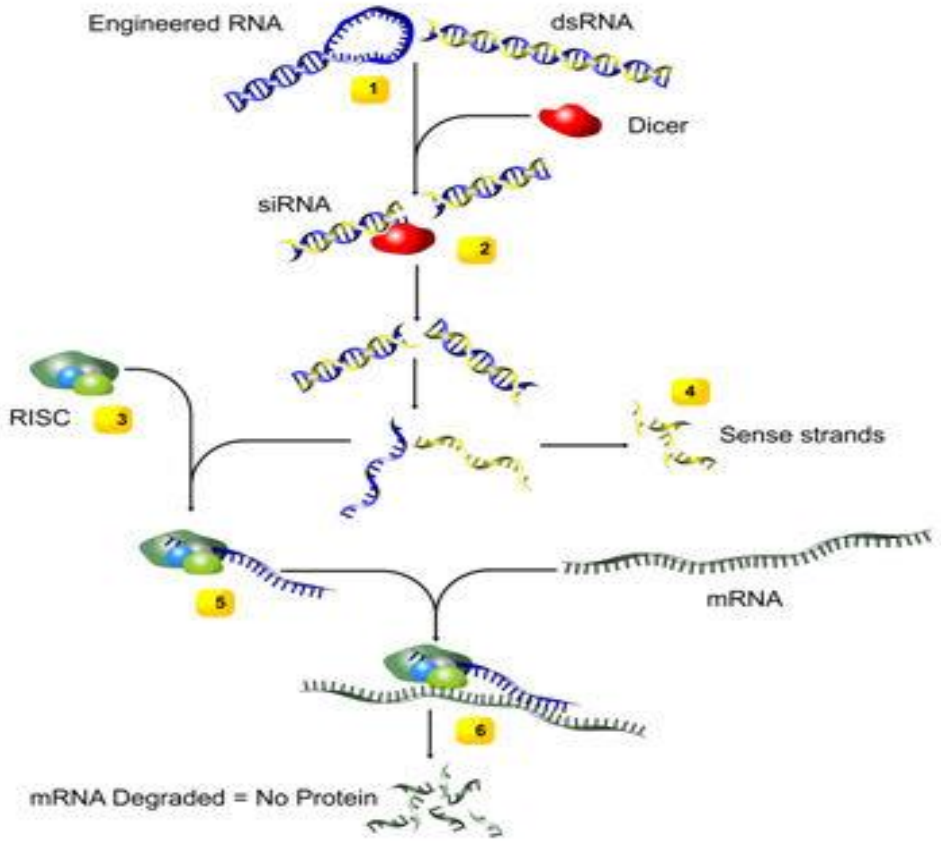

Figure 1. Mechanism of RNAi

Benefits of RNAi

The advantages of RNAi encompass high gene knockdown efficacy, the capacity to accurately hit the gene of interest, and stable and long-term silencing via shRNA expression. This results in a valuable tool being used to resolve a variety of cell biology concerns. Male sterility has indeed been generated via RNAi that is useful in the hybrid seed industry. RNAi can be used to target genes that are only expressed in tissues involved in pollen generation. For example, by suppressing the expression of TA29, a gene required for pollen formation, scientists have created male sterile tobacco strains. In tobacco and tomato, RNAi was employed to alter Msh1 expression, resulting in mitochondrial DNA rearrangements linked to naturally produce cytoplasmic male sterility. Hairpin RNA-expressing vectors, particle bombardment, Agrobacterium-mediated transformation, and virus-induced gene silencing (VIGS) are among approaches that have been used. Plant metabolism has been modified using RNAi to improve nutritional content and reduce toxin generation (Summarized in Table 1). The method takes use of plant RNAi phenotypes that are congenital and enduring.

Table 1. Examples of novel plant traits engineered through RNAi.			
Trait	**Target Gene**	**Host**	**Application**
Enhanced nutrient content	Lyc	Tomato	Increased concentration of lycopene (carotenoid antioxidant)
	DET1	Tomato	Higher flavonoid and b-carotene contents
	SBEII	Wheat, Sweet potato, Maize	Increased levels of amylose for glycemic management and digestive health
	FAD2	Canola, Peanut, Cotton	Increased oleic acid content
	SAD1	Cotton	Increased stearic acid content
	ZLKR/SDH	Maize	Lysine-fortified maize
Reduced alkaloid production	CaMXMT1	Coffee	Decaffeinated coffee
	COR	Opium poppy	Production of non-narcotic alkaloid, instead of morphine
	CYP82E4	Tobacco	Reduced levels of the carcinogen nornicotine in cured leaves
Heavy metal accumulation	ACR2	*Arabidopsis*	Arsenic hyperaccumulation for phytoremediation
Reduced polyphenol production	s-cadinene synthase gene	Cotton	Lower gossypol levels in cottonseeds, for safe consumption
Ethylene sensitivity	LeETR4	Tomato	Early ripening tomatoes
	ACC oxidase gene	Tomato	Longer shelf life because of slow ripening
Reduced allergenicity	Arah2	Peanut	Allergen-free peanuts
	Lolp1, Lolp2	Ryegrass	Hypo-allergenic ryegrass
Reduced production of lachrymatory factor synthase	lachrymatory factor synthase gene	Onion	"Tearless" onion

Applications in Crop Improvement

Plant demand is rising in tandem with population growth, yet food security, malnutrition, and famine are all issues that people are grappling with (Godfray et al. 2010). To address these issues, a combination of genetic engineering, plant physiology, proteomics, and genomics will be required (Mittler and Blumwald 2010; Tester and Langridge 2010). This has been demonstrated with high-yielding cultivars that have improved characteristics (Sharma et al. 2002). The promise of the RNAi technique in crop development has been demonstrated by its contribution to achieving desirable traits by modifying genetic expression. For, instancegrowth of seedless fruits, male sterility and fertility, Biofortification, allergen and toxin removal, therapeutics, altered phenotype, and defence improvement are all examples of improved shelf life and nutritional enrichment.

Prospects for RNAi

According to Godfray et al. (2010), by using genetic engineering and metabolic engineering to design high-yielding crop types, the world can produce a surplus and assure that it is used more effectively. Regarding perennial plant functional genomics and biotechnology, RNAi can be quite useful (Li et al. 2008). Improved crops are made to fulfill global food requirements, fibre, and fuel (Chapotin and Wolt 2007), which will be delivered by developments in RNAi technique. RNAi is utilised as a biological defensive measure versus molecular parasites such as jumping genes and viral genetic elements that impact genome stability, in conjunction to its involvement in regulating the expression. It might be able to silence numerous genes with RNAi by employing a single, well-designed transformation construct. In addition, RNAi can provide broad-spectrum resistance to pathogens with a great standard of diversity, as well as plant stress adaptability. There are numerous applications of RNAi in agriculture science for crop development, such as stress tolerance and nutritional profile enhancement. As a result, RNAi has a large opportunity to profoundly boost agricultural productivity.

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