RNAi and Its Applications in Crop Improvement

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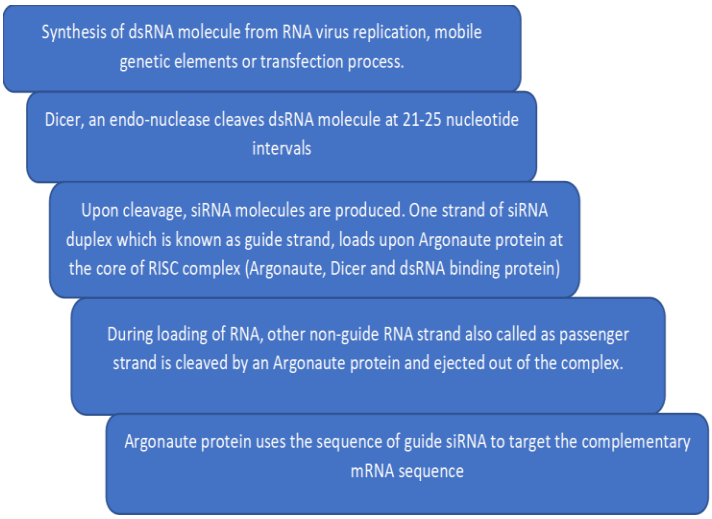
Introduction

RNA interference also abbreviated as RNAi is known by the names such as Post transcriptional gene silencing, or co-suppression. RNAi is a biological process in which small RNA molecules such as siRNA and miRNA inhibit gene expression by targeting mRNA molecule.

In the year 2006, Andrew Z. Fire and Craig C. Mello were awarded the prestigious Nobel prize in Physiology or Medicine for unraveling the mechanism of gene silencing by dsRNA. RNAi studies was done in the model organism, Caenorhabditis elegans (nematode). As an acknowledgement to their contribution, their work was published in the year 1998.

Mechanism of RNAi



Components of RNAi

- ✓ DICER: It is an endoribonuclease belonging to RNA se III family. It cleaves dsRNA into short dsRNA fragments called small interfering RNA (siRNA) of length 20-25 nucleotides usually having two base overhang on 3'end.
- ✓ miRNA: Single stranded RNA molecules of 21-23 nucleotides in length. They partially complementary to one or more mRNA molecules. Their function is to downregulate the gene expression. Active miRNA is produced from native RNA; pri-miRNA is transcribed from chromosomal sequence which is then processed by Drosha into pri-miRNA.
- ✓ siRNA: Small interfering RNA is exogeneous in nature. It is a class of dsRNA molecules of 20-25 nucleotides in length.
- ✓ RNA induced silencing complex: RISC is a multiprotein complex that uses miRNA or siRNA as a template for recognizing and targeting mRNA.

Applications of RNAi in crop biotechnology

Insect resistance

The basic principle in protecting plants against insects through RNAi is by down regulating essential gene functions in the insect, thus resulting in their death. In case of insects the preferred delivery method has been, microinjection of nanogram amounts of double stranded RNA into insect homocoel.

Virus resistance

The effect of gene silencing in plants were first used in efforts to develop resistance to diseases, particularly those caused by viruses. Expression of virus-derived sense or antisense RNA in transgenic plants induce a form of posttranscriptional gene silencing (PTGS).

Fungal resistance

RNA interference is a powerful and versatile genetic tool that can be applied to filamentous fungi of agricultural importance. Gene silencing plays an important role in plant defence against multicellular microbial pathogens; vascular fungi belonging to the *Verticillium* genus.

Table 1: RNAi in fungal resistance

Pathogen	Targeted Region
Magnaporthe oryzae	eGFP
Blumeria graminis f.sp. tritici	Rnr
F. oxysporum f. sp. conglutinans	FOW2, FRP1 and OPR

Abiotic stress

RNA molecules such as miRNA has been involved in drought and salinity stress response, auxin and ABA signalling by carrying out downregulation of target response gene. Down regulation of α-subunit of farsenyltransferase (FTA) enhances the plants

sensitivity towards ABA and drought tolerance in the crop canola.

Quality improvement

Traditional and conventional methods have been used for quality improvement. However, this whole process has been found to be time consuming. Also, limited genetic resources of the crops have very little space for continued improvement. All of this has led to emergence of these techniques which provide a vast amount of scope in quality improvement of crop.

Table 2: RNAi in virus resistance

Species	Order	Crops	Target Gene	Remarks
Diabrotica v. virgifera	Coleoptera	*Zea mays*	*vATPase*	Mortality
Helicoverpa armigera Spodoptera exigua	Lepidoptera	*Nicotiana tabacum*	Nuclear receptor complex of 20-hydroxyecdysone (*HaEcR*)	Molting defect and larval lethality
Helicoverpa armigera	Lepidoptera	*Nicotiana tabacum*	Molt-regulating transcription factor gene (*HR3*)	Developmental deformities and larval lethality
Helicoverpa armigera	Lepidoptera	*Arabidopsis thaliana*	*HaAK*	Developmental Deformities and larval lethality
Myzus persicae	Hemiptera	*Arabidopsis thaliana*	*serine protease*	Progeny reduced

- RNAi mediated ultra-low gossypol cottonseed trait was demonstrated. Gossypol is a terpenoid which is cardiotoxic and hepatotoxic, unfit for consumption. RNAi was used to disrupt gossypol biosynthesis in cottonseed tissue by interfering with the expression of the δ-cadinene synthase gene during seed development., while rest of plant for maintaining capability against insect and diseases. As a result, there was reduction in 99 percent gossypol content
- High lysine corn has been achieved by endosperm specific suppression of lysine catabolism using RNAi approach. Suppression is achieved by expression of an inverted repeat sequence targeting maize bifunctional lysine degradation enzyme- lysine keto glutarate reductase/ saccharopine dehydrogenase (ZLKR/SDH). The suppression of ZLKR/SDH in developing transgenic kernels was directed to endosperm tissue only. Transformation is done through *Agrobacterium* mediated gene transfer.
- RNAi- mediated silencing of three homologues of 1-aminopropane-1-carboxylate (ACS) synthase gene has resulted in improved fruit quality and delayed ripening in tomato. RNAi-ACS fruits were found to bear ~1.5–2.0-fold increase in Titarablesalt. Along with it, 40-45 percent increase in total soluble content was also found.

RNAi for male sterility

- RNAi has also been used to generate male sterility, which is valuable in the hybrid seed industry. Genes that are expressed solely in tissues involved in pollen production can be targeted through RNAi.
- Scientists have developed male sterile tobacco lines by inhibiting the expression of TA29, a gene necessary for pollen development.
- RNAi was also used to disrupt the expression of Msh1 in tobacco and tomato resulting to rearrangements in the mitochondrial DNA associated with naturally occurring cytoplasmic male sterility.

Biosafety concerns

- Potential for off -target caused due to siRNA mediated gene silencing of non-specific target tissue.

- Potential for toxicity due to dsRNA degradation.
- Movement of RNAi molecules is not properly understood.

Conclusion

In recent times, RNAi has emerged out to become as one of the powerful tools for functional genomics. In near future, further elaborate studies will help with better understanding of RNAi mechanism and research in disease treatment. All in all, RNAi has proved itself to be a fascinating technology that can be further exploited for analysis of target genes and gene expression regulation for crop improvement.

References

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Table 3 RNAi in quality improvement

Trait	Target Gene	Host Plant	Potential Benefit
Enzymatic browning	Polyphenyl oxidase gene	Innate Potato	Extended storage lifeReduced acrylamide formation
Maize quality	Starch branching enzyme (Ama-1)	Maize	Up to 50% increase in amylase content
Increased carotenoid and flavinoid content	DET1 gene	Tomato	Consumer health benefits
Reduced ethylene sensitivity	1-Aminocyclopropane-1-carboxylateoxidase (ACC) gene	Tomato	Longer shelf life (slower ripening)
Reduced allergenicity	Arah2 Lolp1, Lolp2	Peanut Ryegrass	Allergen free peanuts Hypo-allergenic ryegrass

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