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RNA Interference in Crop Improvement

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RNA interference (RNAi) is a innovative step in the field of plant molecular genetics and is an incredible revolution in the field of functional genomics. RNA interference includes insertion of match part of the target gene sequence as such no proteins are synthesized and there by blocking the gene function. RNAi inducers, have the potential to effectively silence specific genes which in form of transgenic plants or applied as crop spray. This technology will generate potential for controlling the gene expression. Fire and colleagues, were first to discover RNA interference (RNAi) in the nematode worm *Caenorhabditis elegans*. who found that double-stranded RNA (dsRNA) induced a more potent sequence-specific silencing response than single-stranded antisense RNA alone, Which was customarily used for this purpose. In the recent past with the discovery of small non-coding RNAs which play a important role in RNA silencing RNA-mediated functions has been greatly increased. RNAi operates in plants using double stranded RNA (dsRNA) as a trigger that targets homologous mRNAs for inhibiting its transcription and translation. This RNA-mediated gene control technology has provided new pathways for developing molecular tools for crop improvement by suppressing the genes responsible for various stresses and improving novel traits in plants including disease resistance and will be a promising future therapeutic agent to combat plant invaders.

RNAi was first described in plants as an immune response to viral infection. As early as 1928, it was noticed that as tobacco plants infected with tobacco ringspot virus grew, the upper leaves showed resistance to the effects of the virus. It is now known that dsRNA intermediates produced during virus infection activate the RNAi machinery to silence expression of complementary genes, thus producing immunity to the virus. This defense against foreign genetic material is one of several physiologic pathways that are induced by

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naturally occurring dsRNAs in a wide variety of eukaryotic organisms including fungi, plants, and animals. With some variations, these responses are all mediated by a common RNAi pathway that involves processing of the dsRNA into short duplexes of about 22 base pairs with characteristic end structure.

Mechanism of RNAi:

RNA interference refers collectively to diverse RNA based processes that all result in sequence specific inhibition of gene expression at the transcription, mRNA stability or translational level. The unifying features of this phenomena are the production of small RNAs (21.26 nucleotides) that act as specific determinants for down-regulating gene expression and the requirement for one or more members of Argonaute family of protein. RNA i operates by triggering the function of dsRNA intermediates, which are processed into RNA duplexes of 21-24 nucleotides by ribonuclease III Like enzyme called Dicer (Mehrotra and Aggarwal 2003). Once produced, these small RNA molecules of short interfering RNAS (SiRNAs) are incorporated in a multi-subunit complex called RNA induced silencing complex (RISC). The RISC complexis formed by an endonuclease and siRNA among other component. The siRNAS within RISC acts as a guide to target the degradation of complementary messenger RNAS (mRNAS), When dsRNA molecules produced during viral replication trigger gene silencing the process is called Vinas-induced gene silencing (VGS). One interesting feature of RNA silencing in plants is that once it is triggered in a certain cell, a mobile signal is produced and spread through the whole plant causing the entire plant to be silenced. This silencing process is also enhanced by the enzymatic activity of the RISC complex, mediating multiple timer reaction. Furthermore, production of the more secondary siRNAs leads to enrichment of silencing via its spread from the first activated cell to neighboring cells, and systematically through system . The cell-to-cell spread can be mediated as passive spread of the small RNAS via plasmodesmata, since it does not spread into meristematic cells. The discovery of RNA binding protein (PSRPI) in the phloem and its ability to build 25 nts.

How RNA Works:

The entry of long double stranded RNA, such as an introduction of a rogue genetic element or a viral intruder, triggers the RNA pathway of cells. This results in the recruitment of the Dicer. The Dicer leaves the dNA into 20-25 basepairs long, fragments, called small

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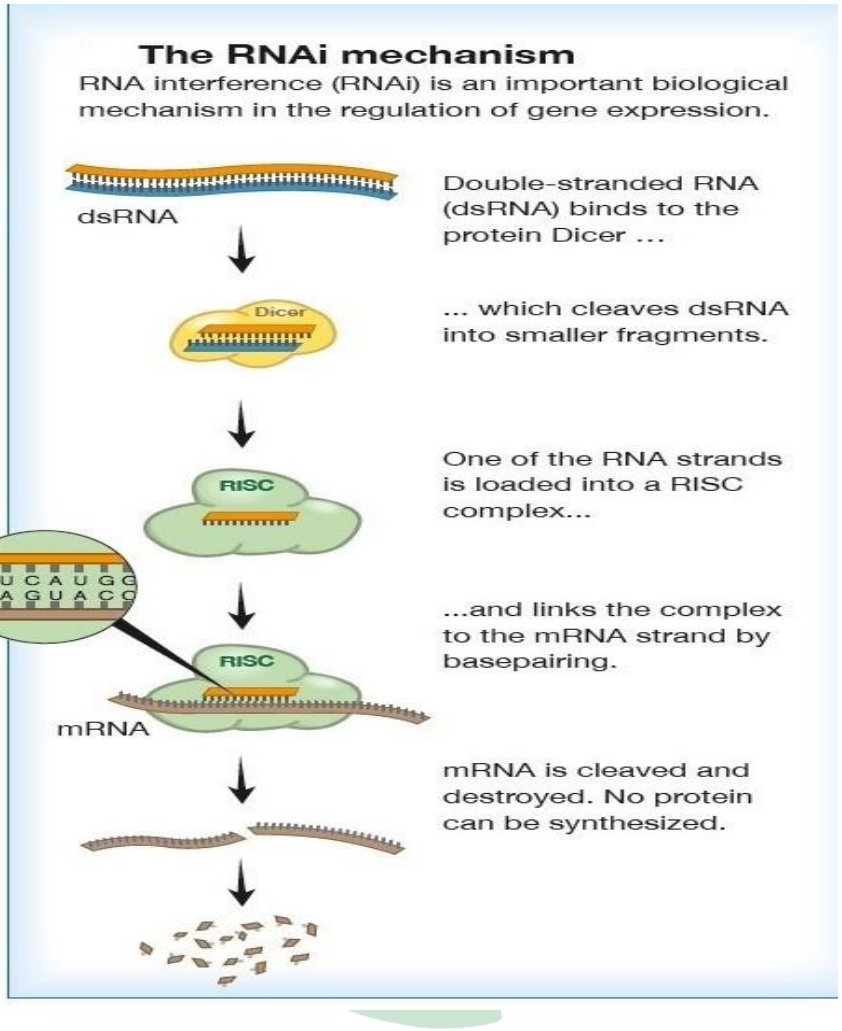
interfering RNA (siRNA). An RNA-induced silencing complex (RISC) then distinguishes as sense or antisense between the two siRNA strands. The sense strands which has exactly the same sequence as of the target gene are degraded. The antisense strand on the other hand are incorporated to the RISC also used as guide to target messenger RNA (mRNA) in a sequence-specific manner, Messenger RNAS (mRNA), which codes for amino acids, are cleaved by RISC. The activated RISC can repeatedly participate in mRNA degradation, inhibiting protein synthesis.



Disease Management by RNAi:

The RNA interference technology has emerged as one of the most potential strategies for enhancing the building of resistance in plants to combat various fungal, bacteria, viral and nematode diseases causing huge losses in important agricultural crops (Singh, 2005) The nature of this biological phenomenon has been evaluated in a number of host-pathogen systems and effectively used to silence the action or pathogen. Many of the examples listed

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below illustrate the possibilities for commercial exploitation of the inherent biological mechanism to generate disease resistant plants in the future by taking advantage of this approach eg: including: *Cladosporium fulvum, Magnaporthe oryzae, Venturia inequali, Neurospora crassa* and *Fusarium graminearum* whether it is suitable for large-scale mutagenesis in fungal pathogens remains to be tested. Such silencing mechanisms (RNAi) can also be exploited to manage and protect plants from viral infections. The effectiveness of this technology in generating virus-resistant plants was first reported to PVY in potato, harboring vectors for simultaneous expression of both sense and antisense transcripts of the helper-component *proteinase* (*HC-Pro*) gene

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. For instance, 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.

Disease Management by RNAi:

For RNAi application in agricultural pest control the target insect must take the dsRNA autonomously from the transgenic plants expressing dsRNA. the feeding by the insect should be continuous as the insects lack an amplification mechanism based on RdRP. Many of the agricultural pest species have already been targeted by RNAi technology using various genes and delivery methods. However three orders have been the major focus of the development of transgenic plants expressing target gene region they are lepidoptera coleoptera and hemiptera. The table describes provides the examples of the effect of the RNAi in these orders

Specie	Order	Crop	Target Gene	Effect
Diabrotica v. virgifera	Coleoptera	*Zea mays*	*vATPase*	Mortality
Leptinotarsa decemlineata	Coleoptera	*Solanum tuberosum*	*β-actin, Shrub*	Mortality
Helicoverpa armigera Spodoptera exigua	Lepidoptera	*Nicotiana tabacum*	Nuclear receptor complex of 20-hydroxyecdysone (*HaEcR*)	Molting defect and larval lethality

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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 and Nicotiana benthamiana*	*MpC001, Rack1*	Progeny reduced

Limitations of RNAi:

- The limitations mainly depend on the type of polymerase ultimately used to recognize and amplify the siRNA sequence.
- even following the recommended rules for siRNA design does not ensure effective silencing of the target gene.
- The efficacy of siRNA-mediated suppression of gene expression depends on a number of factors, including not only the chosen siRNA sequence but also the structure of the siRNA, and the receptiveness of the cell type to siRNA uptake.
- the half life of the target message and/or protein needs to be considered in order to achieve optimal silencing.
- Although siRNAs are relatively stable in cell culture conditions, they require enhanced nuclease and thermodynamic stability when in circulation in vivo.

Conclusion:

Because the benefit of RNAi applications to both basic and applied research is substantial, strategies must be developed to maintain a high level of siRNA efficiency while minimizing the potential for the misinterpretation of data due to the nonspecific or off-target effects that have been associated with siRNA expression. Finally, as our understanding of this fascinating mechanism of gene regulation improves, new ways to effectively harness RNAi for experimental and therapeutic approaches will be revealed.