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RNAi and its Applications in Crop Improvement

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Abstract

RNA interference (RNAi) is a powerful tool for gene silencing in different organisms, including plants. It is being used in functional genomics to decipher the function of genes. This technology has also witnessed a variety of potential applications in agriculture for crop improvement, including the development of crops for resistance against biotic (weeds, pathogens, insect pests, and nematode parasites) and abiotic stresses (drought, high and low temperature, etc.), nutritional quality improvement, healthier oils, delayed ripening, male sterility, modification of flowering time and flower color, alteration of plant architecture, enhancement of secondary products, and removal of allergens and toxins

1. Introduction

RNAi or Post-Transcriptional Gene Silencing (PTGS) is highly conserved and sequence-specific gene silencing mediated by double-stranded RNA (dsRNA). A similar sequence-specific gene silencing was reported in Petunia, which is called Co-suppression and Quelling (or PTGS) in a fungus (*Neurospora crassa*). 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 was discovered by Andrew Fire and Craig Mello in *Caenorhabditis elegans* in 1998 (Fire et al., 1998) and they received Nobel Prize in Physiology or Medicine in 2006 for this important breakthrough discovery.

It is a mechanism that inhibits gene expression by hindering the transcription of specific genes or at the stage of translation and it is one of the important reverse genetics approaches in which resistance against diseases, insects, and abiotic stresses is developed. In functional genomics, this allows down-regulation in gene expression with more accuracy without interfering expression of other genes and finally leads to yield improvement.

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1.1. What is RNA Interference?

It is a mechanism that blocks the central dogma by inhibiting the expression of m-RNA without altering the existing DNA or gene sequence by forming double-stranded RNA. It silences the gene through different methods like complete removal or degradation of mRNA. It blocks expression at the transcription level and also at the translation level.

1.2. Mechanism of RNA Interference

The mechanism of gene silencing is broadly divided into two major steps: the initiation step and the effector step. In the initiation step, the enzyme Dicer, a member of the RNAse III family of dsRNA – specific ribonucleases, cleaves the dsRNA molecule to 21-23 bp double-stranded fragments known as short interfering RNAs (siRNAs) or guide RNAs. In the effector step, the duplex siRNA is then unwound by the helicase activity associated with a distinct multiprotein complex known as RNA-induced silencing complex or RISC. The siRNA strand that is complementary to the targeted mRNA is then used as a primer by an RNA-dependent RNA polymerase (RdRp) to convert the cognate mRNA into dsRNA itself. This dsRNA form of mRNA then becomes a substrate for Dicer cleavage activity, which leads to the destruction of the mRNA and the formation of new siRNAs (Figure 1). This step effectively amplifies the RNAi response and creates a self-perpetuating cycle of degradative polymerase chain reaction that will persist until no target mRNAs remain. This basic core pathway defines the RNAi response as one of the most elegant and efficient biochemical mechanisms in nature.

2. Applications of RNA Interference in Crop Improvement

2.1. Manipulation of plant architecture using RNAi

When non-transgenic scions are grafted upon transgenic rootstock that silenced the *CmMET1* gene there was an early floral phase in the chrysanthemum due to reduction in expression levels of *CmMET1* (DNA methyltrasferase1) in scions grafted onto transgenic chrysanthemum and increase in the expression of the *CmDRM2* (methyltransferase) gene. Suppression of *CmMET1* may result in shorter plants and early blooming (bloomed eight days earlier) (Li et al., 2019).

2.2. Manipulation of flower color

Anthocyanin biosynthesis facilitated by downregulation

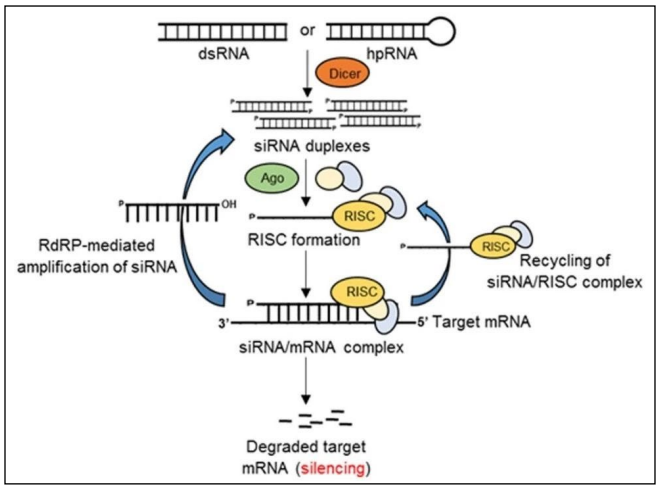

Figure 1: Schematic diagram of RNAi-mediated gene silencing in eukaryotes (Majumdar et al., 2018)

of *chalcone synthase* (CHS) genes has resulted in manipulating flower color in many plant species, and another enzyme, chalcone isomerase (CHI), is also involved in the flavonoid biosynthetic pathway, catalyzing the synthesis of chalcone into flavanone in plant cells.

2.3. Development of seedless fruit

It is feasible to produce coreless and seedless apples by suppressing *AGAMOUS*-like carpel identity genes and hormone-induced fruit sets. *AGAMOUS* suppression resulted in an increase in petal whorls and a complete absence of carpel formation (Ireland et al., 2021). MAPK regulates the vegetative and reproductive development of plants. Down or up regulation of *SlMPK4* expression did not influence tomato vegetative development. RNAi-mediated inhibition of *SlMPK4* disrupts the pollen development resulting in a very low fruit set and *SlMPK4*-suppressed lines produce seedless tomato fruit even though without pollination (Wang et al., 2022).

2.4. Quality improvement

In transgenic soybean seeds, oleic acid content increased significantly (20% to 80%), but linolenic and linoleic acid content decreased when the *GmFAD2-1B* gene's expression was down-regulated by a seed-specific promoter of the β-conglycinin alpha subunit gene (Yang et al., 2018).

2.5. Altering metabolic pathways

Plants having medicinal properties have been utilized to treat various human illnesses for centuries. These medicinal chemicals might be overexpressed in plants. Overexpressed or silenced *MsYABBY5*-introduced

transgenic plants were created by using RNAi and the transgenic lines revealed that reduced *MsYABBY5* expression resulted in higher terpene levels, whereas overexpression resulted in lower terpene levels.

2.6. Insect resistance

RNAi technology has also been utilized to control insect pests to reduce crop loss. Numerous current findings have focused on host-induced gene silencing, in which many RNAi-based transgenes have been produced and evaluated as proof of concept for insect pest control, as RNAi has already revealed a huge potential for the control of insect pests. The creation of a new generation of insect pest control products might be aided by the siRNA pathway. Developing a brand-new generation of insect-resistant plants involves feeding dsRNAs as a diet, which helps to efficiently down-regulate the targeted genes in insects (Cagliari et al., 2019; Mishra et al., 2021). One such technique is the delivery of double-stranded RNA (dsRNA) as a BioClay spray to manage the sap-sucking insect whitefly. Multiple stages of the whitefly life cycle are disrupted in Planta adjuvant-enhanced foliar absorption complementing clay-mediated dsRNA delivery (Jain et al., 2022)

2.7. Phytopathogenic resistance

Sm-AMP-D1 gene sequence codes the plant defensins such as anti-microbial peptides inhibiting the growth and development of plant pathogens. Transgenic banana with overexpression of *Sm-AMP-D1* showed enhanced resistance against *Fusarium oxysporum f. sp. cubense* compared to non-transgenic banana plants (Ghag et al., 2014). When compared to untransformed wild-type strains of *Fusarium oxysporum* using the hpRNA construct of *Pbs2*, *Hog1* and *Fmk1* signaling genes, transformants exhibited a considerable reduction in pathogenesis in tomato seedlings. (Pareek and Rajam, 2017)

2.8. RNAi in abiotic stress resistance

RNAi was involved in abiotic stress response, including osmoprotectant, ABA response, antioxidant, and auxin signaling by down-regulating the target genes of the abiotic stress responses. Transgenic lines with suppressed *BrDST71* gene resulted in more drought stress tolerance in *Nicotiana tabacum* (Park et al., 2018). RNAi-mediated suppression of *OsABA8ox1* resulted in slight increase in endogenous ABA levels resulting in enhanced tolerance of rice plants to saline-alkaline stress (Liu et al., 2022).

3. Conclusion

RNAi-mediated gene silencing takes place at the post-transcriptional level without involving the host plant's protein machinery and producing no transgenic protein. It is quite improbable that these RNAi plants would produce allergenicity and toxicity in the absence of transgenic protein, the target pathogens/pests would develop resistance to RNAi and there could be few bio-safety concerns. The siRNAs produced from the expressed long dsRNA can have off-target effects, i.e., silencing of unintended or non-target genes if there is a substantial homology.

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