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Role of RNA Interference (RNAi) in Plant Disease Management Strategies - A Review

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ABSTRACT

One of the most important limiting factors of agriculture is plant diseases caused by various pathogens like fungi, bacteria, viruses and nematodes. Exclusive dependence on chemicals is leading to health issues and of late the problem of resistance in pathogens. RNA interference (RNAi) is a novel strategy for control of various pathogens through silencing of genes associated with pathogenesis. Different methods of introducing dsRNA in to cells are Agroinfiltration, Micro bombardment and Virus Induced Gene Silencing VIGS. History of RNAi and implications of RNAi technology in management of various plant pathogens like fungi, bacteria, viruses and nematodes are briefly reviewed.

Key words: *RNA interference (RNAi)-History-Fungi-Bacteria-Viruses-Nematodes*

INTRODUCTION

One of the most important limiting factors of agriculture is plant diseases caused by various pathogens like fungi, bacteria, viruses and nematodes. These are generally managed by using chemicals either to kill them or their vectors. But the extensive use of chemicals is causing lot of ecological damage often killing the beneficial organisms along with the pathogens. Apart from this exclusive dependence on chemicals is leading to health issues and of late the problem of resistance in pathogens is very alarming.

Modern technologies such as transcriptomics, proteomics, and metabolomics are now proved to be important in understanding metabolic pathways and the role of key genes associated with their regulation. Identification of important genes required for the invasion, growth and pathogenesis of various pathogens and specific silencing of such genes is very effective for management of various pathogens in crop plants. RNA interference (RNAi) is a novel strategy for control of various pathogens through silencing of genes associated with pathogenesis. Plants expressing dsRNA targeting essential genes in pathogens can provide resistance to various pathogens by silencing the genes responsible for the disease in pathogens.

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RNAi gene silencing at posttranscriptional has been reported by various workers and has become a choice for gene targeting in different pathogenic groups like fungi (Nakayashiki, 2005), viruses (Baulcombe, 2004), bacteria (Escobar *et al*., 2001), nematodes (Kharkas, 2008).

BRIEF HISTORY OF RNAi:

The history of RNAi has been well elucidated by Sharma *et al.* (2013). The evolutionary story of RNAi began in the early 1990s with Napoli and colleagues in an effort to deepen the purple colour by a gene chalcone synthase observed that the pigmentation in the flowers of transformed plants was not enhanced but rather depigmented (Napoli *et al*., 1990). Based on the fact that both the transgene and the endogenous gene were suppressed, the observed phenomenon was termed "co-suppression". This phenomenon remained undeciphered at that time as post-transcriptional gene silencing (PTGS) was not known (Napoli *et al*., 1990; Jorgensen *et al*., 1996; Cogoni and Macino, 2000). This phenomenon of suppression of an endogenous gene by transformation with homologous sequences was also observed in the fungus *Neurospora crassa* where it was termed quelling (Romano and Macino, 1992). However, the significance of these observations went unnoticed for several years until the mystery was solved in 1998, when it was demonstrated that dsRNA is even more effective in silencing gene expression than antisense RNA, the phenomenon was termed as RNAi (Fire *et al*., 1998). Based upon these and other findings initially made in studies of plants, RNAi has evolved as a mechanism to defend plant cells against fungal, bacterial, viral and nematode infections (Mann *et al*., 2008).

THE ESSENCE OF RNAi:

The delivery of double-stranded RNA (dsRNA) into an organism or cell, will induce a sequence-specific mRNA degradation mechanism that effectively silence a targeted gene. Silencing by dsRNA is known by different names like Post transcriptional gene silencing (PTGS), Quelling and RNA interference (RNAi).

COMPONENTS OF RNAi:

The mechanism of RNAi was well described by Khraiwesh *et al*., (2012) with 4 major components *viz.*, dsRNA (Trigger), Dicer (Processor), siRNA/mi RNA (Processor products), RNA-Induced Silencing Complex (RISC) (Effector complex).

dsRNA (trigger):

The process of RNAi starts with the presence of long dsRNA corresponding to a particular gene acting as trigger for the pathway. These are often designed and introduced by the researchers or produced as a result of infection by the pathogen as a means of natural defense mechanism.

Dicer (Processor):

Dicer is an endoribonuclease in the RNase third family that cleaves double stranded RNA (dsRNA) and pre-micro RNA(mi RNA) into short double stranded RNA fragments called small interfering RNA(si RNA) about 20-25 nucleotides. Dicer facilitates the formation of the RNA-induced silencing complex (RISC), whose catalytic component argonaute is an endonuclease capable of degrading messenger RNA (mRNA).

Small interference RNA (siRNA) & MicroRNA (miRNA):

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 21-26 base pairs in length. miRNAs are small RNAs , originate from endogenous miRNA genes of an organism. They are known to regulate a variety of processes including growth, development and response to abiotic and biotic stresses including during host microbe interactions .

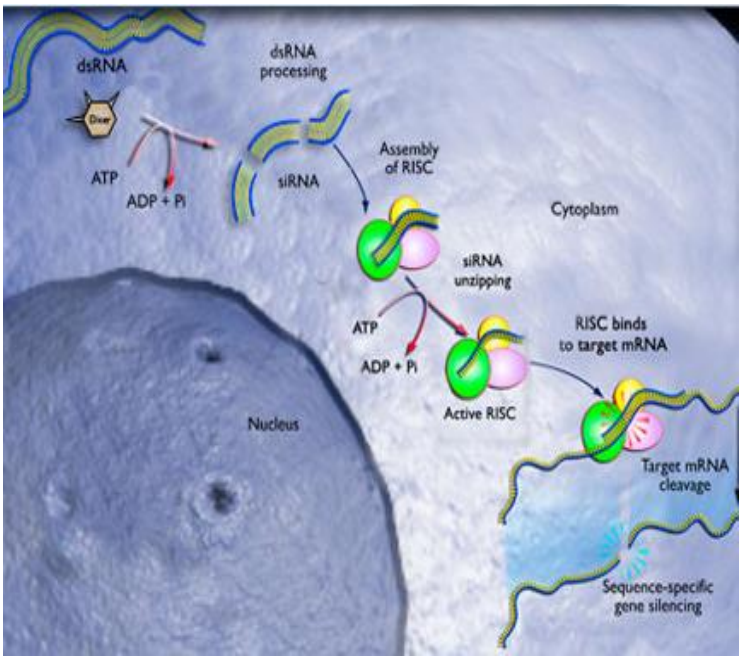
RNA Induced Silencing Complex, or RISC:

RNA Induced Silencing Complex, or RISC, is a multiprotein complex that incorporates one strand of a small interfering RNA (siRNA) or

micro RNA (miRNA).The active components of an RNA induced silencing complex (RISC) are endonuclease called argonaute proteins, which cleave the target mRNA strand complementary to their bound siRNA. RISC uses the siRNA or miRNA as a template for recognizing complementary mRNA When it finds a complementary strand, it activates Rnase and cleaves the mRNA.

MECHANISM OF RNAi:

RNAi operates by triggering the action of dsRNA intermediates, which are processed into RNA duplexes of 21-24 nucleotides by a ribonuclease III like enzyme called Dicer. Once produced, these small RNA molecules or short interfering RNAs (siRNAs) are incorporated in a multi subunit complex called RNA induced silencing complex (RISC). RISC is formed by a siRNA and an endonuclease among other components. The siRNAs within RISC acts as a guide to target the degradation of complementary messenger RNAs (mRNAs) and inhibiting the respective gene (Wani *et al.*, 2010).



Schematic representation of steps in RNAi

METHODS TO INDUCE RNAi IN PLANTS:

Most reliable and commonly used approaches for delivery of dsRNA to plants cells are Agroinfiltration, Micro bombardment and Virus Induced Gene Silencing VIGS.

Agroinfiltration: Agroinfiltration is a powerful method to study processes connected with RNAi.The injection of Agrobacterium carrying similar DNA constructs into the intracellular spaces of leaves for triggering RNA silencing is known as agroinoculation or agroinfiltration. In most cases agroinfiltration is used to initiate systemic silencing or to monitor the effect of suppressor genes (Hilly and Liu, 2007).

Micro Bombardment : In this method, a linear or circular template is transferred into the nucleus by micro bombardment. Synthetic siRNAs are delivered into plants by biolistic pressure to cause silencing of GFP expression. Bombarding cells with particles coated with dsRNA, siRNA or DNA that encode hairpin constructs as well as sense or antisense RNA,

activate the RNAi pathway (Klahre *et al.*, 2002).

Virus Induced Gene Silencing (VIGS): Modified viruses as RNA silencing triggers are used as a mean for inducing RNA in plants. Different RNA and DNA viruses have been modified to serve as vectors for gene expression. Some viruses, such as Tobacco mosaic virus (TMV), Potato virus X (PVX) and TRV, can be used for both protein expression and gene silencing (Timmermans *et al.*, 1994).

APPLICATIONS OF RNAi IN PLANT DISEASE MANAGEMENT:

RNAi technology has emerged as one of the most potential and promising strategies for enhancing the building of resistance in plants to combat various fungal, bacterial, viral and nematode diseases causing huge losses in important agricultural crops. There has been innumerable number of applications of RNAi in developing resistant varieties to various pathogenic groups. A few representative examples are discussed below.

Management of Plant Pathogenic Fungi:

RNA mediated gene silencing (RNA silencing) is used as a reverse tool for gene targeting in fungi. Homology based gene silencing induced by transgenes (co suppression), antisense, or dsRNA has been demonstrated in many plant pathogenic fungi, including *Cladosporium fulvum, Magnaporthae oryzae , Venturia inaequalis, Neurospora crassa, Aspergillus nidulans,* and *Fusarium graminearum* (Wani *et al.,* 2010). Development of Host induced RNAi system in wheat stripe rust fungus *Puccinia striiformis* f. sp. *tritici* has been reported by Chuntao *et al.*, (2011). *Puccinia striiformis* f. sp. *tritici* gene fragments PSTha12J12 coding for secreted protein during pathogenesis were delivered into plant cells through Barley Stripe Mosaic Virus system for initiating the RNAi silencing signals crossed the barriers of host cell membrane and extra haustorial matrix and silencing is triggered in haustorial cells. In Barley powdery mildew caused by *Blumeria graminis,* the function of *Mlo* gene in Barley codes for negative regulator of race-non specific resistance was explained using dsRNA bolistic application. Plants having mutated *Mlo* alleles have complete resistance due to abortion of penetration of fungal hyphae (Schweizer *et al.* 2000).

Management of Plant Pathogenic Bacteria:

In Crown gall disease expression of iaaM and ipt oncogenes is necessary for tumourogensis (gall formation). Expression of these genes was targeted using RNAi constructs and transgenic *Arabidopsis thaliana* and *Lycopersicon esculentum* transformed with RNAi constructs, targeting iaaM and ipt gene(s) showed resistance to crown gall disease (Escobar *et al.* 2001). The natsiRNA (nat-siRNAATGB2) was strongly induced in *Arabidopsis* upon infection by *Pseudomonas syringae pv tomato* and down-regulates a PPRL gene that encodes a negative regulator of the RPS2 disease resistance pathway. As a result, the induction of nat-siRNAATGB2 through RNAi mechanism increased the RPS2-mediated race-specific resistance against *P. syringae* pv *tomato* in *Arabidopsis* (Agarwal, *et al.,* 2006).

Management of Plant Pathogenic Viruses:

RNAi technology for virus resistant plants was first reported to PVY in potato by simultaneous expression of both sense and antisense transcripts of the helper component proteinase (HC Pro) gene (Waterhouse *et al.,* 1998). The utilization of RNAi technology has resulted in inducing immunity reactions against several other viruses in different plant-virus systems (Wani, *et. al.,* 2010). Multiple suppressors have been reported in the Citrus tristeza virus, where p20 and coat protein (CP) play important roles in suppression of the silencing signal, and p23 inhibited intracellular silencing. In case cassava brown streak virus disease (CBSD) caused by the Ipomoviruses viz., Cassava brown streak virus (CBSV) and Ugandan Cassava brown streak virus (UCBSV) where RNAi with small interfering

RNAs (siRNAs) from coat protein (CP) sequence was used all non transgenic control plants developed CBSD symptoms whereas the transgenic lines were healthy (Ogwok *et al.*, 2012).

Management of Plant Parasitic Nematodes:

RNAi is used against plant parasitic nematodes belonging to the genera *Meloidogyne, Heterodera, Globodera* by targeting plant genes that are involved with the infection process and/or the essential genes within the nematode. Genes coding for production of enzymes necessary for pathogenesis like Cysteine proteinases, Dual oxidase, Splicing factors, Integrase, C type lectin, Major sperm protein, Aminopeptidase, β1, 4 Endonuclease, Chitin synthetase, Pectate lyase are targeting by providing ds RNA by inducing through oral ingestion by second-stage juveniles using octopomine and resorcinol- and serotonin. The genes targeted by RNAi to date are expressed in different tissues and cells in intestine, reproductive system, sperms and oesophageal glands etc. Uptake of dsRNA from the gut after ingestion is a proven route in many nematodes and later the RNAi becomes systemic in the body. Depending up on the genes coded RNAi will result in management of nematodes by delay in egg hatching, reduction in established nematode population, increasing male female ratio, reduction in invasion capacities, decreased motility etc. (Wani *et al.*, 2010).

SUMMARY

RNAi mediated crop protection is very interesting and novel method which is highly efficient and stable. RNA interference (RNAi) is a novel strategy for control of various pathogens through silencing of genes associated with pathogenesis. Plants expressing dsRNA targeting essential genes in pathogens can provide resistance to various pathogens by silencing the genes responsible for the disease in pathogens. The disease management is brought about in a highly regulated and environmentally less risky compared to other management strategies like chemicals. RNAi technology is more stable as the risk of development of resistance by pathogens is meager. Apart from this RNAi offers enormous scope for studying gene functions at a faster pace so that the genes responsible for pathogenesis are quickly identified and silenced using complementary dsRNA. Hence, RNAi technology can be considered to be safe, ecofriendly and ever green and offers great leverage in human efforts of combating various fungal, bacterial viral and nematode diseases of crops.

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