REVIEW

UNLEASHING THE POWER OF RNA INTERFERENCE (RNAI) TECHNOLOGY: REVOLUTIONIZING PEST AND PLANT DISEASE CONTROL IN THE COCOA INDUSTRY

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ABSTRACT – *In disease control management, RNA interference (RNAi) technology has emerged as an eco-friendly alternative to the conventional use of pesticides and fungicides in agriculture. RNA interference (RNAi) is a mechanism for post-transcriptional silencing triggered by the presence of double-stranded RNA (dsRNA), leading to the degradation of mRNAs (target genes) that exhibit complementarity to the dsRNA. Prolonged use of pesticides raises concerns regarding environmental sustainability, human health, and the emergence of fungicide/pesticide-resistant pests or plant diseases. Therefore, many studies have demonstrated the significant potential of RNAi technology in controlling plant diseases, as the application of double-stranded RNA is known to inhibit the development and growth of target pests or pathogens and reduce the expression of specific target genes. Despite the promising applications of RNAi in agriculture, its extensive utilization in the cocoa industry for controlling major pests and plant pathogens, such as Conopomorpha Cramella, Phytophthora Palmivora, and Oncobasidium theobromae, remains largely unexplored. Thus, this review provides insight into the mechanism and modes of application of RNAi technology. Additionally, it discusses existing studies on the use of RNAi in the agriculture sector and the limited exploration within the cocoa industry. The paper concludes by addressing the challenges associated with the adoption of RNAi technology in pest and disease management.*

Keywords: RNA interference, plant disease control, cocoa industry, cocoa pest, cocoa plant disease

INTRODUCTION

In the present day, the use of pesticides and fungicides continues to be a prevalent practice among most farmers in Malaysia to prevent the attack of plant disease or pests which will risk their crops production. According to Kamaruzaman *et al*. (2020), the utilization of pesticides in Malaysia amounted to 49,199.43 tonnes of active ingredients, equating to an average application rate of 5.9 kg per hectare of crop land. In the cocoa industry, application of pesticides is common practice adopted by the cocoa growers to boost the cocoa productivity and prevent pest attack (Miyittah *et al*., 2022). For instance, it is reported that deltamethrin, alphacypermethrin, cypermethrin and chlorpyrifos are the insecticides that are primarily used to control cocoa pod borer. However, frequent use of chemical pesticides increases the chance of pesticide resistance because of overuse, raises production costs, and puts the grower's health and the environment at danger (Bakar, S., 2016).

Miyittah *et al*., (2022) have conducted an assessment on the pesticide exposure risks among the cocoa farmers in Western region of Ghana and the result showed that 66% of them experienced health symptoms such as headaches, burning eyes, skin rashes, itching and chest pain. This is probably due to the extensive use of pesticides for pest control combined with either improper or non-use of personal protective equipment (PPE) at different phases of pesticide use. Furthermore, based on the outcome by Shuklan *et al*. (2023), exposure of cypermethrin may harm both human and mammals as cypermethrin with doses of 25, 50, and 75 mg/kg led to adverse effects on both male and female albino rats. Few symptoms that can be observed in the rats are loss of body weight, decreased food consumption, vomiting and excessive salivation. Moreover, pesticide residue or pesticides constitute a significant cause of water pollution. This occurrence can be attributed to the direct application of pesticides for the control of aquatic flora or through agricultural runoff (Farag *et al*., 2021). Additional research (Dawar *et al*., 2016; Paravani *et al*., 2018, 2019) has demonstrated that cypermethrin can trigger genotoxicity and oxidative stress in zebrafish (Danio rerio) exposed to it, and also lead to malformations in rohu (*Labeo rohita*) during early developmental stages. Therefore, it can be inferred that prolonged exposure to pesticides may have adverse effects on humans, non-target animals, and the environment.

Due to the long-term effects of pesticide and fungicide usage which is concerning, there is a growing

demand for alternative solutions. RNA interference (RNAi) technology has emerged as a recent and eco-friendly approach in pest control management. This technology induces post-transcriptional gene silencing, meaning that its integration into the target organism inhibits the expression of specific target genes responsible for the development and pathogenicity of the organism. Furthermore, RNAi technology has attracted considerable attention from researchers due to its great specificity and efficiency (de Oliveira Filho *et al*., 2021). Numerous studies reported the positive results of the application of RNAi on target organisms. For example, Qiao *et al*., (2023) have applied the double-stranded RNA (dsRNA) encapsulated in chitosan and designed to target gene of G Protein-Coupled Receptor Kinase 2 (*GRK2*) in *Apolygus lucorum*. Their studies showed that the dsRNA effectively suppressed the expression of the G protein-coupled receptor kinase 2 gene by 70%, resulting in a significant increase in mortality by 50%, a reduction in weight by 26.54%, and an extended developmental period by 8.04%. Not only that, Sundaresha *et al*. (2022) reported that the multigene-targeted dsRNA treatments (combination of sorbitol dehydrogenase, heat shock protein 90, translation elonga- tion factor 1-α & combination of phospholipase-D like 3 and glycosylphosphatidylinositol-anchored acidic serine–threonine-rich HAM34-like protein) able to reduce mycelium growth of the plant pathogen, *Sclerotinia sclerotiorum* as well as its spore production significantly.

In the past few decades, the RNAi technology has been explored extensively for plant disease control. Nevertheless, its application in the cocoa industry is not yet evident, and there is a scarcity of related studies in this context. Therefore, exploring the application of RNAi technology in the cocoa industry becomes imperative, as it has the potential to serve as the optimal alternative for managing cocoa pests and pathogens. This approach could significantly enhance sustainable agriculture and maximize cocoa production. This review will explore RNAi technology in the cocoa industry, focusing on its mechanisms and modes of application, and will address the benefits and challenges associated with its use.

MECHANISM OF RNA INTERFERENCE (RNAi)

Basically, RNA interference (RNAi) is recognized as a conserved and fundamental component of gene regulation mechanisms found in all eukaryotes, which operates through small RNAs (sRNAs) that facilitate gene silencing at the post-transcriptional level. This post-transcriptional gene silencing initiated when double stranded RNA (dsRNA) is incorporated into the target organism and further cleaved into short 21–24 nucleotide (nt) small-interfering RNA (siRNA) duplexes by an RNaseIII-like enzyme called Dicer as shown in Figure 1. Double-stranded siRNAs associate with Argonaute (AGO) proteins to form an RNA-induced silencing complex (RISC) and initiates the unwinding of the siRNA. This process results in the generation of an antisense (or guide) strand that forms base pairs with mRNA target sequences that are complementary. The last step will be recognition of the target sequence by the RISC complex. The target RNA molecules will be degraded and unable to be further transcripted or translated (Degnan *et al*., 2023; Werner *et al*., 2020).

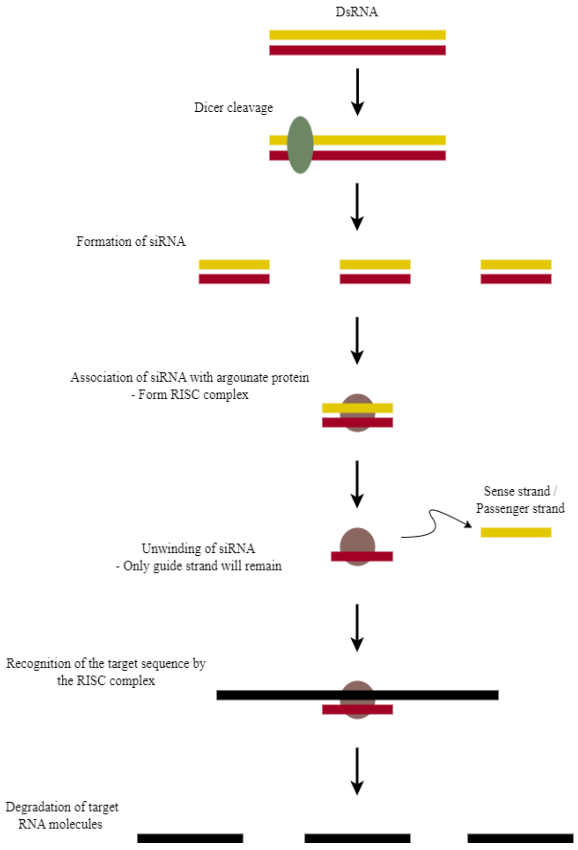

Figure 1: Mechanism of RNA interference (RNAi)

MODES OF APPLICATION OF RNA INTERFERENCE (RNAi)

There are two primary strategies for disease control based on RNA interference (RNAi): host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS). However, there are certain differences between these approaches as portrayed in Table 1.

Table 1: Comparison of HIGS and SIGS approaches

Host-Induced Gene Silencing (HIGS)	Spray-Induced Gene Silencing (SIGS)
Involve the genetic modification of the plant itself to express small RNAs that target the specific genes.	Involve exogenous application (spray / topical delivery) of RNA with specific target sequence.
Involve creation of transgenic plants which are mediated by *Agrobacterium tumefaciens* / gene gun.	Does not involve creation of transgenic plants.

Host-induced gene silencing (HIGS):
In the context of HIGS, plants undergo transformation by introducing hairpin RNA (hpRNA) containing transgenes that share homology with specific genes in pathogens. Typically, the target gene is cloned into a binary vector before being transformed into *Agrobacterium tumefaciens*. Subsequently, the transfer of the target gene into the plant genome is facilitated by *vir* genes encoded on the Ti plasmid of *A. tumefaciens*. Successful integration of the target genes into the plant genome, with subsequent expression in plant cells, results in the development of transgenic plants (Yildiz *et al*., 2016). When the target gene is expressed as double-stranded RNA molecules in the plant cell, it undergoes further cleavage into small interfering RNA (siRNA). The guide strand of siRNA is exported to the target pest or fungi, where it induces gene silencing through complementary base pairing, effectively suppressing the expression of the target gene in the pathogen (Bowen *et al*., 2022).

Numerous researchers have explored the efficacy of HIGS for enhancing crop protection. Within the context of this review paper, Table 2 presents compelling evidence of the beneficial outcomes associated with HIGS implementation in pest and plant pathogen management. Notably, studies conducted by Walker *et al*. (2023), Wang *et al*. (2016), and Thakare *et al*., (2017) have demonstrated the efficacy of RNAi constructs introduced into host plants via *Agrobacterium tumefaciens*, leading to successful expression within the transgenic host plants. These constructs effectively silenced targeted genes of specific fungi, resulting in significant reductions in lesion size and expression levels of the targeted genes. Besides that, the application of HIGS on plant pests has yielded significant results, as evidenced by research from Faisal *et al*. (2019), Adeyinka *et al*., (2023), and Chatterjee *et al*., (2022). Through feeding bioassays on transgenic plants, these studies demonstrated substantial downregulation of targeted genes and increased mortality rates among the target pests.

Spray-induced gene silencing (SIGS):
SIGS typically involves applying dsRNA externally through spraying or topical delivery onto plant surfaces. Upon uptake by fungi or pests, this dsRNA triggers the target organism's RNA interference machinery, leading to the silencing of specific genes without involving any gene modifications on the plant cells (Ghosh *et al*., 2023). Additionally, as for fungal cells, there are two possible routes of entry of the dsRNA molecules, as reported by de Oliveira Filho *et al*. (2021). The first involves their ingress into plant cells, where they utilize the plant's silencing machinery to generate siRNA molecules, subsequently migrating to the fungus. The second pathway entails direct absorption of dsRNA molecules by the fungus, recruiting the pathogen's own cellular machinery.

Similar to HIGS, SIGS has shown promising results as a biopesticide and biofungicide, as demonstrated by various studies presented in Table 3. Research by L. Qiao *et al*. (2023) and Sarkar & Roy-Barman (2021) has shown that the application of dsRNA leads to reduced lesion size on leaves infected by target fungi. Additionally, reduced disease severity has been observed on dsRNA-sprayed infected leaves and fruits (Cao *et al*., 2024; McRae *et al*., 2023; L. Qiao *et al*., 2023), along with abnormal conidiation of fungi (Sarkar & Roy-Barman, 2021). Sarkar & Roy-Barman (2021) reported significant downregulation of the target gene *MoDES1*, an important defense suppressor in rice, following dsRNA feeding treatment using CM agar.

Moreover, SIGS has proven effective against target pests. Studies by Willow *et al*. (2023), Rodrigues *et al*. (2021), and X. Wang *et al*. (2023) have demonstrated a substantial reduction in the expression of target genes upon application. X. Wang *et al*. (2023) observed abnormal pupation and emergence rates of *Tuta absoluta* after feeding on dsRNA-sprayed tomato leaves. In terms of mortality, Rodrigues *et al*. (2021) noted larval mortality after 6 hours of dsRNA exposure, with greenhouse trials showing no surviving insects 14 days post-treatment. However, Willow *et al*. (2023) reported contradictory outcomes, with targeted pollen beetles surviving after 11 days of dsRNA treatment. This discrepancy may be attributed to the lower concentration of dsRNA used, potentially insufficient to induce lethal levels for effective pest control.

POTENTIAL OF RNA INTERFERENCE (RNAi) FOR PEST AND DISEASE CONTROL IN COCOA INDUSTRY

RNA interference research has been extensively explored for pest and disease control in agriculture. However, its application in the cocoa industry faces severe limitations. The cocoa sector faces serious challenges from three main pests and diseases: *Conopomorpha cramella*, *Phytophthora palmivora*, and *Ceratobasidium theobromae*, which negatively affect cocoa productivity. As shown in Table 5, a recent study by Tan *et al*. (2019) focused on utilizing RNA interference to control the infestation of *Conopomorpha cramella*, the cocoa pod borer. The study demonstrated that dsRNA targeting specific genes like aminopeptidase, calreticulin, and cathepsin L resulted in a higher mortality rate compared to the control group (treated with water only) and led to a significant decrease in gene expression. Furthermore, another study has been made by Tan *et al*. (2021) to identify suitable target genes for effective gene silencing of Cocoa Pod Borer. Through transcriptome analysis, they identified several genes involved in biological processes within the insect such as choline dehydrogenase, NAD(P)-dependent dehydrogenase and WD40 repeats. Genes involved in reproduction, including *porin, dsx* and *fruitless* are found as well in the insect, where these genes are crucial for mating behaviour, spermatogenesis and sex determination, respectively. In contrast, research on the efficacy of RNA interference for controlling *Phytophthora palmivora* and *Ceratobasidium theobromae* in cocoa have not been undertaken to date. However, studies have been conducted to identify genes responsible for the pathogenicity and developmental processes of these fungi. As illustrated in Table 4, prior research predominantly concentrates on the identification of promising target genes within cocoa pests and fungi to facilitate efficient gene silencing in preparation for subsequent applications.

Transcriptomic profiling of *C. cramella, P. palmivora*, and *C. theobromae* revealed that the highly expressed genes are associated with pathogenicity and the developmental processes of these insects or fungi. For instance, a study by Masanto *et al*. (2021) identified four actively expressed genes in the model plant *Nicotiana benthamiana* infected by *Phytophthora palmivora*. These genes include *CRN1*, *Pec1*, *Pec3*, and *RXLR5*. The *CRN1* gene is responsible for inducing crinkling and necrosis, while the RXLR effector acts as both an activator and suppressor of plant immunity, inhibiting the secretion of immune protease and triggering cell death in the host plant. Additionally, *Pec1* and *Pec3*, known as pectinase genes, play a crucial role in breaking down pectin, a component of the plant's primary cell wall and middle lamella, which can be found in the cocoa pod husk.

In the study conducted by Ali *et al*. (2019), a transcriptome analysis of RNA extracted from cacao stems and petioles infected by *Ceratobasidium theobromae* was carried out, in comparison with *Rhizoctania solani*. Their findings showed the pivotal role of several gene categories in the onset of Vascular Streak Dieback (VSD), notably Carbohydrate-Active enzymes (CAZy) genes, KEGG-pathway associated genes, and putative effector proteins. CAZy genes are involved in the modification and degradation of cell wall components and other organic substances, while KEGG-pathway associated genes play specific roles in biological pathways. Additionally, putative effector proteins are important in suppressing plant defense mechanisms.

Consequently, by designing dsRNA to target and silence these genes associated with pathogenicity and development pathways, it is anticipated that the growth of the target pests and fungi will be prevented under normal conditions. This approach holds the potential to substantially diminish the severity of diseases affecting cocoa pods.

Based on the findings of transcriptome analysis, the integration of RNA interference technology in the cocoa industry should be promptly pursued as it holds significant promise for advancing sustainable agricultural practices. Table 5 showed the utilization of RNA interference, which can be associated with the cocoa industry. As for *Conopomorpha cramella*, the efficacy of RNAi has been tested on adult CPB, resulting in high CPB mortality rates and the suppression of target gene expression. While RNA interference studies targeting *Phytophthora palmivora* and *Ceratobasidium theobromae* remain scarce, studies related with application of RNA interference on target organisms, which are closely related to those cocoa-affecting-fungi, have been included in Table 5. It can be seen that all of these studies utilizing RNA interference technology to target genes which are crucial for development and growth of those fungi and led to significant results.

Through detached leaf assay, it is observed that there is reduction of lesion size (Chen *et al*., 2022; Ivanov & Golubeva, 2023; Tiwari *et al*., 2017) as well as sporulation (Kalyandurg *et al*., 2021; Sundaresha *et al*., 2022) on leaves treated with dsRNA targeting specific genes compared to the control group. Furthermore, morphological observations by Sundaresha *et al*. (2022) and Tiwari *et al*. (2017) demonstrate decreased disease severity and delayed

disease progression on infected leaves treated with dsRNA. Gene expression analysis is a crucial method for validating successful gene silencing in RNA interference. As depicted in Table 5, most studies have conducted gene expression analyses, except for Ivanov & Golubeva (2023). These studies consistently report significant downregulation of target genes following the application of dsRNA, whether applied exogenously or endogenously. Hence, these promising results indicate that RNA interference technology has the potential to effectively control both *P. palmivora* and *C. theobromae* as well, necessitating more research into its application to these fungi.

CHALLENGES / LIMITATION IN RNAI DEVELOPMENT

Selection of target genes:

Despite positive outcomes of application of RNA interference which applied in either HIGS and SIGS, there are some challenges or limitations that need to be addressed in the development of RNAi-based control. Primarily, the efficacy of RNA interference may be compromised by the selection of target genes. According to Singewar & Fladung (2023) and Zhang *et al*. (2023), the ideal target gene for RNA interference must be essential for pest survival and highly expressed during early development of the insect. Application of dsRNA targeting genes which are crucial for the development and growth of the pest must lead to a decrease in the transcript level and eventually, causing mortality of the pest. The phenomenon occurs when target gene expression is inhibited, preventing the pest from developing and growing normally.

In order to ensure successful gene silencing against plant pathogens, it is important to target genes that are either essential for virulence or play critical roles in disease progression. By targeting these genes, the expected results include a decrease in their expression levels, hence delaying disease progression in infected plants. For instance, a study by Kalyandurg *et al*. (2021) has tested application of RNA interference to target one of the genes called *OSBP* gene. Although the exact function of *OSBP* is not clearly established, in other eukaryotes it is suggested to play a role in membrane-mediated lipid transport and intercellular distribution of lipid molecules. Thus, their outcome showed that there is an approximately twofold decrease in *PiOSBP* transcript levels when treated with dsRNA targeting that gene, however, the disease progression on the infected leaves is observed and not significantly inhibited. It can be concluded that the decrease in transcript levels of *OSBP* is insufficient to impact on *P. infestans* infection. Hence, this is why screening of RNAi targets is necessary to ensure effective RNAi. Initially, RNAi targets are selected based on the discovery of key genes in other organisms or by cDNA library screening. Due to availability of second-generation sequencing, expression profiling and transcriptome reconstruction of numerous insects can be conducted and lead to efficient identification of potential target genes (Zhang *et al*., 2022).

Stability of dsRNA for field application:

Another significant challenge in the development of RNAi-based biopesticides for field applications is the stability of RNAi molecules. Studies by Mann *et al*. (2023) and Zhang *et al*. (2022) highlight the high possibility of dsRNA degradation due to environmental factors such as sunlight and rain. For instance, San Miguel and Scott (2016) (as cited in He *et al*. (2022) demonstrated dsRNA degradation on agarose gel following 30 minutes of UV exposure. Additionally, L. Qiao *et al*. (2023) found that dsRNA encapsulated within artificial nanovesicles remained on leaves after rinsing, unlike naked dsRNA, which showed a drastic reduction in fluorescence as observed through confocal laser scanning microscopy.

To combat environmental degradation, incorporating dsRNA into carrier molecules like nanoparticles has shown promise (K. Wang *et al*., 2020). This approach not only protects dsRNA but also enhances its stability and cellular uptake. For example, encapsulating dsRNA targeting the glycerol-3-phosphate dehydrogenase (*G3PDH*) gene of the rice striped stem borer (*Chilo suppressalis*) with nanoparticles significantly improved dsRNA stability and efficacy. Feeding assays revealed a marked reduction in *G3PDH* expression in larvae fed encapsulated dsRNA compared to those fed the control gene (*GFP* gene). Thus, using suitable carrier molecules that are cost-effective, non-toxic, and environmentally friendly can significantly enhance the stability and effectiveness of RNAi-based biopesticides.

Cost effectiveness of dsRNA production:

Another limitation of RNAi development is the cost-effectiveness of synthesized dsRNA. Effective RNAi delivery requires large quantities of dsRNA, but commercially available RNAi kits are typically expensive and produce limited amounts of dsRNA, making them efficient only for small-scale screening processes. For instance, it is estimated that approximately 10 grams of dsRNA per hectare are needed for field-scale management of pests and pathogens. In vitro dsRNA transcription systems incur a minimum cost of $100 per gram of dsRNA, which is not cost-effective for large-scale applications. However, microbial and cell-free based dsRNA production methods have been developed, significantly reducing production costs to approximately $2 per

gram of dsRNA (Dalakouras *et al*., 2024; Mann *et al*., 2023). For example, in an in vivo feeding bioassay, a study by Sharif *et al*. (2022) demonstrated that bacterially expressed dsRNA targeting the acetylcholinesterase (*AChE*), ecdysone receptor (*EcR)*, and v-ATPase-A (*vAA*) genes led to reduced mRNA levels of these target genes, resulting in mortality and abnormal development in larvae of *Helicoverpa armigera.*

Off-target effects:

Last but not least, RNA interference (RNAi) technology is known for its specificity since the dsRNA is solely designed to inhibit the expression of particular genes that have specific sequences for the management of pests and pathogens. On the other hand, another issue to consider is that the administration of dsRNA, particularly when done externally, may have off-target effects on non-target organisms. Although the specific data needed for regulatory decision-making for biocontrol products based on externally applied dsRNA is unclear, it is almost certain that information on the application's possible risks to people, animals, and the environment will be needed (Raybould & Burns, 2020).

The research carried out by Pampolini & Rieske (2020) examined the off-target effects that resulted from applying dsRNA targeting particular genes in the Emerald Ash Borer, using various model insects. These included the Colorado Potato Beetle (herbivore), Spotted Lady Beetle (predator), Eastern Subterranean Termite (detritivore), and European Honeybee (pollinator), pivotal for considering further deployment in field settings. The targeted genes encompassed the heat shock 70-kDa protein (*hsp*), shibire (*shi*), and U1 small nuclear ribonucleoprotein (*rnp*).

Results indicated notably low larval mortality percentages following dsRNA application targeting these genes in herbivore, predator, and detritivore larvae, compared to those targeted with a positive control gene (*ATPase*). Consequently, successful emergence into adult stages was observed in these insects. With the exception of the pollinator larvae, no observable decrease in relative expression was found in the *hsp* gene expression analysis following dsRNA exposure in any of the model insects.

In the case of the pollinator, significant decreases in relative expression of *hsp*, *shi*, and *rnp* were noted on day 10 post-ingestion of dsRNA. Despite this, larval survival remained largely unaffected, with mortality rates below 30%. In silico evaluation utilizing the NCBI nucleotide BLAST tool detected sequence homologies for *shi* and *rnp* in honeybees when queried against available predicted sequences. One potential explanation for these findings is the potential oversaturation of the RNAi machinery, possibly affecting insect immune responses, particularly following exposure to a single high concentration of dsRNA.

Additionally, the study extended its assessment to classical biological control agents, namely *T. planipennisi* and *S. galinae*. Interestingly, no notable reduction in gene expression of the target genes was observed in these agents compared to the positive control gene. This underscores the importance of conducting thorough bioinformatics analyses prior to the development of RNAi-based biocontrol methods, alongside comprehensive risk assessments on non-target organisms, before considering field application and commercialization.

Table 2: Overview of HIGS research on targeting specific organisms and genes

References	Target Organisms	Target Genes	Transgenic plant production	Results
Walker *et al*. (2023)	*Sclerotinia sclerotiorum* (Fungi)	*ABHYDROLASE-3* – Involved in polyamine biosynthesis during S. sclerotiorum infection	Transgenic Arabidopsis	Reduced *S. sclerotiorum* lesion size, fungal load, and *ABHYDROLASE-3* transcript abundance in developed transgenic *Arabidopsis thaliana* (AT1703).
M. Wang *et al*. (2016)	*Botrytis cinerea* (Fungi)	*DCL1* & *DCL2* – Involved in regulating the growth, conidiation and pathogenicity of the fungi	Transgenic Arabidopsis	Exhibited much smaller lesions and less fungal growth after *B. cinerea* infection.
Thakare *et al*. (2017)	*A. parasitus* (Fungi)	Aflatoxin - Toxic secondary metabolites	Transgenic maize plants	No detection of aflatoxin in the kernel of the transgenic maize after pathogen infection and the gene expression of aflatoxin in transgenic maize is at very low level.
Faisal *et al*. (2019)	*Myzus persicae* (Pest)	Acetylcholinesterase 1 (*Ace 1*) - Work as neurotransmitters in insect	Transgenic tomato plants	A significant downregulation of *Ace 1* is observed in aphids fed on transgenic plant with construct T-452 (double insert) as well as reduction in the number of aphids nymphs produced per adult at both 22℃ and 27℃.
Adeyinka *et al*. (2023)	*Chilo partellus* (Pest)	Chitinase - Assists in the breakdown of chitin present in both the exoskeleton and peritrophic membrane (PM).	Transgenic maize plants	Feeding of transgenic maize plants led to lower relative growth rate of larvae, reduced mRNA expression of chitinase gene and higher mortality rate.
Chatterjee *et al*. (2022)	*Maruca vitrata* (Pest)	Alpha-amylase - Involved in the digestion process and catalyses the hydrolysis of starch and glycogen.	Transgenic pigeonpea	Feeding of transgenic pigeonpea led to reduced leaf damage, one-fold decrease in the transcript accumulation as well as larvae mortality.

Table 3: Overview of SIGS research on targeting specific organisms and genes

References	Target Organisms	Target Genes	Incorporation of Nanocarrier	Results
Sarkar & Roy-Barman (2021)	*M. oryzae* (Fungi)	*MoDES1* - Innate defense suppressor in rice, plays a crucial role in detoxifying reactive oxygen species (ROS) generated by the plant and is essential for the progression of pathogenesis.	-	Abnormal conidiation (reduced growth, deformed conidia) was observed. The target gene found to be downregulated as there were defects in invasive hyphal growth on onion epidermal peel and reduced lesion size on rice leaves following treatment with dsRNA.
L. Qiao *et al*. (2023)	*Botrytis cinerea* (Fungi)	*DCL1* & *DCL2* – Involved in regulating the growth, conidiation and pathogenicity of the fungi.	Artificial nanovesicle: lipid-based nanoparticle	Inhibited *Botrytis cinerea* virulence on tomato fruits, grape berries, lettuce leaves and rose petals as reduced lesion size observed.
McRae *et al*. (2023)	*Golovinomyces orontii MGH1* (Fungi)	*CYP51* - Required for the synthesis of sterol, a component of fungal cell membranes.	-	Led to reduction of spore production and in visible powdery mildew disease on the dsRNA-sprayed *A. thaliana* plant.
Cao *et al*. (2024)	*Erysiphe Quercicola* (Fungi)	β-tubulin – Important for various cellular functions.	-	Led to reduced disease severity of powdery mildew as well as expression level on dsRNA treated leaves compared to those treated with water.
Willow *et al*. (2023)	Pollen beetle (Pest)	Coatomer subunit α (*αCOP*) – Essential eukaryotic gene	-	Led to decrease in αCOP mRNA expression but no effect on the survival rate of the pollen beetle after treatment with dsRNA.
Rodrigues *et al*. (2021)	Colorado potato beetle (Pest)	*PSMB5* – A subunit of the proteasome, which is an essential cellular machinery responsible for protein degradation.	-	Exposure to dsRNA for 6 h caused larval mortality and decreased target gene expression. Subsequent greenhouse trials revealed that no insects survived beyond 14 days following the treatment.
X. Wang *et al*. (2023)	*Tuta absoluta* (Pest)	*kr-h1* - Mediates the actions of the two critical hormones of insects, the juvenile hormone (JH) and 20-hydroxyecdysone (20E).	Star Polycation (SPc)	Reduced the expression of target gene, pupal weight and emergence rate as well as exhibit no effect on the non-target organism.

Table 4: Overview of research focusing on the identification of promising target genes within cocoa pests and fungi for efficient gene silencing

References	Target Organisms	Target Genes	Function of Target Genes
Masanto *et al*. (2021)	*Phytophthora palmivora*	*CRN1*	Induce crinkling and necrosis.
		Pec1 and *Pec3*	Breaking down pectin which is a component of plant primary cell wall and middle lamella.
		RXLR5	Can be activator and suppressor of plant immunity by preventing the secretion of the immune protease and triggering cell death on the host plant.
Ali *et al*. (2019)	*Cerobasidium theobromae*	Carbohydrate-active enzymes (eg: *CE8, GH28, AA9, GH16*)	Involved in modification and degradation of cell wall and other organic matter.
		KEGG-pathway associated genes	Involved in biological pathway.
		Putative effector proteins	Suppress the plant immunity.
Tan *et al*. (2021)	*Conopomorpha cramella*	NAD(P)-dependent dehydrogenase	Involved in insect metabolism, where it catalyses reversible conversion of various alcohols in larval feeding sites to their corresponding aldehydes and ketones, thus contributing to detoxification and metabolic purposes.
		GTPase	Regulate diverse cellular and developmental events.
		WD40 repeat genes	Involved in biological processes.

Table 5: Application of RNA interference on target organisms - Cocoa Pod Borer and organisms which are closely related to those cocoa-affecting-fungi

References	Target Organisms	Target Genes	Mode of Application	Results
Tan *et al*. (2019)	*Conopomorpha cramella*	*Amp* – Enhance immune response *Crt* – Contribute to feeding process *Cpl* – Involved in development, growth and metamorphosis	SIGS – feeding	The feeding of dsRNA led to a higher mortality rate of adult CPB, ranging from around 60% to 90%, and resulted in a reduction of target gene expression, with decreases ranging from 10 thousand to 23 million-fold compared to the control group.
Sundaresha *et al*. (2022)	*Phytophthora infestans* (same genus as *Phytophthora palmivora*)	*Hsp90* - Chaperone protein that assists other proteins to fold properly. *EF-1 α* – Vital for protein synthesis & cell proliferation. *SDH* – Important for sorbitol metabolism and colonization of plant tissues. *PLD-3* - Invade the host by damaging its cell membrane. *GPI-HAM34* - Involved in incorporation into the cell wall.	SIGS - Spray	5 days after treatment. combination of multigene dsRNA which encapsulated with or without nanoclay led to significant reduction in sporulation (without nanoclay: 1×10^3; with nanoclay: no sporulation) and disease severity (without nanoclay: 10%; with nanoclay: 4%) compared to control group (sporulation count: 12.5×10^3; disease severity: 73.3%). Furthermore, a decrease in the expression of the target genes was observed in plants treated with dsRNA.
Kalyandurg *et al*. (2021)	*Phytophthora infestans* (same genus as *Phytophthora palmivora*)	*PiGPB1* - Important for signal transduction during pathogenesis and proper sporangial development. *PiHmp1* – Vital for intercellular progression and host colonization. *PiCut3* – Involved in degradation of cutin at the outermost pathogen - plant barrier. *PiEndo3* – Important for host tissue invasion.	SIGS - Spray	Suppressed sporulation significantly (reaching levels of up to approximately 90%), with the exception of gene *Hmp1*, and also hindered fungal development in comparison to the control group. A reduction in the relative gene expression of target genes was also observed.

Table 5: Application of RNA interference on target organisms - Cocoa Pod Borer and organisms which are closely related to those cocoa-affecting-fungi

References	Target Organisms	Target Genes	Mode of Application	Results
Ivanov & Golubeva (2023)	*Phytophthora infestans* (same genus as *Phytophthora palmivora*)	*Inf4* – Involved in sterol transport. *Inf1* - Involved in plant immune system modulation, causing tissue necrosis at a late stage of infection.	SIGS – Applied exogenously on leaves / nutrient media.	All plants treated with dsRNA (rank of leaf lesion = more than 4.9), whether administered via leaf application or root uptake, exhibited notable protection against late blight when compared to the control group (rank of leaf lesion = 1.4)
Tiwari *et al*. (2017)	*Rhizoctonia solani*	*PMK1* - Responsible for fungal appressorium, cuticle penetration and viability inside the host plant.	Host induced - biolistic particle gun used for gene delivery into host plant.	Led to downregulation of the target gene, reduction of lesion size and delayed disease development in the transgenic rice plant compared to non-transgenic rice plant.
Chen *et al*. (2022)	*Rhizoctonia solani*	*CAT* – Counteract pro-oxidants generated by plants. *CRZ1* – Important for cellular processes. *PG* – Involved in pectin cleavage, causes cell separation, and macerates host tissues.	SIGS	The expression of target genes was decreased by approximately 31% to 44%, and the lesion size on infected maize leaves was reduced by over 50%. However, no significant alterations in mycelial growth characteristics on the PDA medium were observed across all treatments.
L. Qiao *et al*. (2021)	*Rhizoctonia solani*	*DCTN1* – Important for intracellular transport. *SAC1* – Regulate actin dynamics, which are essential for various cellular processes such as cell division, cell shape maintenance, vesicle trafficking, and motility. *PG* – Important for pectin degradation	Topical application	Resulted in a notable decrease in the relative biomass of *R. solani* by approximately 50% compared to the control group. Additionally, the expression of target genes in rice leaves treated with dsRNA was reduced by more than 60% compared to the control group.

CONCLUSIONS

RNA interference (RNAi) technology emerges as a promising eco-friendly alternative to conventional pesticides and fungicides in agriculture, offering a revolutionary approach to plant disease control within the cocoa industry. The specificity and efficiency of RNAi have garnered significant interest among researchers, with numerous studies showcasing its efficacy in targeting specific organisms. However, challenges persist in the widespread adoption of RNAi, particularly concerning the cost-effective production of double-stranded RNA (dsRNA) for large-scale applications. Current commercially available RNAi kits are limited in quantity and expensive, posing a barrier to extensive utilization. Moreover, the application of RNAi technology in the cocoa industry for managing major pests and plant pathogens like *Conopomorpha Cramella*, *Phytophthora Palmivora*, and *Oncobasidium theobromae* remains largely unexplored, underscoring the necessity for further research and exploration in this domain.

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