Review Article

ROLE OF SMALL RNAs IN PLANT IMMUNITY

ABSTRACT: Small RNAs play a crucial role in the regulation of gene expression, operating at both the transcriptional and post transcriptional stages. These molecules have the ability to initiate target destruction or block translation. The activation of plant immunity is frequently associated with the increased expression of growth-regulatory microRNAs (miRNAs) following the detection of pathogens. Additionally, conserved miRNAs play a crucial role in regulating auxin signalling. Plants are capable of generating two distinct categories of short RNA molecules, namely microRNAs and small interfering RNAs. Three highly prevalent miRNA families induce the generation of secondary siRNAs from 74 out of the 79 NLR transcripts that produce siRNAs. This phenomenon highlights the appeal of employing secondary siRNA-mediated control as a viable technique. Two microRNAs (miRNAs) derived from plants have been found to target virulence genes in the fungal disease *Verticillium dahliae*. Additionally, it has been observed that secondary small interfering RNAs. siRNAs can increase plant protection by facilitating host-induced gene silence. TasiRNAs have been observed to exhibit enhanced mobility and resilience in the context of non-cell-autonomous silencing. The production and function of short RNAs in fungal and oomycete infections have been minimally investigated. The production of small RNAs (sRNAs) by *Verticillium dahliae*, a fungal pathogen, has been observed to occur in a size range of 18-25 nucleotides (nt) without any specific size preference. Additionally, a specific microRNA (miRNA) known as VdmilR1, measuring 21 nt in length, has been thoroughly studied and its functional characteristics have been elucidated. *Phytophthora* species, which are filamentous eukaryotic microbes, have been found to inflict substantial economic losses in the fields of agriculture and forestry. RNA interference (RNAi) has the potential to regulate the expression of neighbouring effector genes by inducing the creation of heterochromatin. In plants, the movement of small RNAs has been observed both locally and across extended distances. These small RNAs have the potential to enhance plant defense mechanisms against nonviral diseases by acting as systemic signaling molecules. The domain of sRNAs in plant-pathogen/parasite interactions has experienced significant advancements; nonetheless, numerous obstacles remain in the realm of trans-species gene silencing that require more investigation.

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Keywords: sRNA, miRNA, siRNA, pathogen, plant defense, effector genes and signal molecules.

1. INTRODUCTION

The cellular processes in eukaryotes are extensively influenced by RNA interference (RNAi) mediated by short RNAs (sRNAs). This regulatory mechanism involves the suppression of target genes by various mechanisms such as transcriptional repression, transcript breakage, or inhibition of translation [1,2]. Small RNA-mediated silencing is a quantitative process that lends itself well to the precise adjustment of gene expression. This mechanism also facilitates systemic responses to environmental signals and allows for the regulation of target genes within intimate symbiotic relationships [3,4]. sRNA-mediated regulation assumes a distinctive and significant role in the context of plant-pathogen interactions, since it bestows precise temporal and spatial controls. Plants possess a highly developed and resilient immune system that is dependent on the identification of microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs) [5,6]. Pattern-triggered immunity (PTI) serves as a crucial defense mechanism against the colonization of various possible pathogens. However, it is worth noting that certain infections have developed the ability to overcome this protection by employing virulence proteins known as effectors [7, 8, 9]. Numerous filamentous pathogens have the ability to generate suppressors of RNA silencing, so exemplifying a dynamic competition focused on the defense mechanism of small RNA-mediated silencing. Plants have developed an additional layer of protection as a counteractive strategy, which relies on the identification of pathogen effectors by the nucleotide-binding leucine-rich repeat (NLR) receptors. The activation of NLR immune receptors leads to the induction of effector-triggered immunity (ETI), a process frequently accompanied by programmed cell death aimed at limiting pathogen dissemination. The process of gene silence is a widely observed mechanism of gene control in eukaryotes. Eukaryotic pathogens utilize small RNAs (sRNAs) to modulate their pathogenicity [10,11,12]. The growing body of data suggests that small RNAs (sRNAs) have a role in the development of diseases by regulating gene expression within an organism and across different species. This regulatory mechanism is

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particularly relevant in the context of nonviral diseases caused by filamentous pathogens, such as fungi and oomycetes [13].

2.Plant Immunity by Small RNAs

2.1Biogenesis and Function of Small RNAs in Plants

Plant small RNAs (sRNAs), which are usually composed of 20-24 nucleotides, play a crucial role in regulating gene expression at both the transcriptional and posttranscriptional stages [14, 15]. Transcriptional gene silencing (TGS) can be facilitated by the mediation of RNA-directed DNA methylation or histone methylation. Additionally, they have the ability to induce target degradation or block translation by forming pairs with messenger RNAs (mRNAs). Plant short RNAs (sRNAs) can be categorized into two primary groups, namely microRNAs (miRNAs) and small interfering RNAs (siRNAs). MicroRNAs (miRNAs) are derived from single-stranded precursors and undergo processing by the ribonuclease Dicer-like 1 (DCL1), which belongs to the RNase III family. This processing results in the production of miRNA/miRNA duplexes [16, 17]. The duplexes undergo methylation by the enzyme HUA ENHANCER1 (HEN1) in order to safeguard the 3′ terminal nucleotide against destruction. One of the two strands in the duplex, known as the miRNA strand, is incorporated into ARGONAUTE (AGO) proteins. These proteins assemble into RNA-induced silencing complexes (RISC) and execute gene-silencing activities at the post-transcriptional gene silencing (PTGS) level [17]. Small interfering RNAs (siRNAs) are generated from double-stranded RNAs (dsRNAs) that possess a perfect match in their complementary sequences. These dsRNAs are produced by RNA-dependent RNA polymerases (RDRs) [14]. The generation of small interfering RNAs (siRNAs) can be induced by exogenous RNA molecules or through the targeted cleavage of certain endogenous transcripts under the guidance of microRNAs (miRNAs). Secondary small interfering RNAs (siRNAs), alternatively referred to as phased siRNAs [18], are generated at regular intervals of 21 or 24 nucleotides. The secondary siRNA pathway possesses functional significance due to its potential for inducing extensive gene silencing through a regulatory cascade, wherein genes can be silenced by both primary miRNA and siRNAs. Secondary small interfering RNAs (siRNAs) have the potential to exert their regulatory effects on genes that are not under the control of primary microRNAs (miRNAs), hence broadening the scope of gene silencing mechanisms [19].

2.2 Regulation of Defense by miRNA

The induction of the defense response in plants is frequently accompanied with a trade-off in terms of growth, and microRNAs (miRNAs) play a crucial role in governing plant growth and development. The activation of plant immunity is frequently associated with an increase in the expression of growth-regulating microRNAs (miRNAs) following the detection of pathogens. The miRNAs that respond to pathogens serve as regulators to maintain a delicate equilibrium between defense and growth, by modulating the signaling pathways of phytohormones [20, 21, 22]. The conservation of microRNAs (miRNAs) plays a crucial role in the regulation of auxin signalling, which exhibits an antagonistic relationship with the salicylic acid-mediated defensive mechanism against pathogens that feed on viable host cells [39]. One example of a microRNA that demonstrates this behavior is MiR393, which specifically targets the TIR1/AFB auxin co-receptors and is upregulated in response to treatments involving MAMP/PAMP. Plants are capable of generating two distinct categories of short RNAs (sRNAs), namely microRNA [miRNA] and small interfering RNA (siRNA). Both microRNAs (miRNAs) and small interfering RNAs (siRNAs) undergo methylation by the enzyme HEN1. These methylated RNA molecules play a crucial role in regulating the expression of target genes through two mechanisms: transcript cleavage and translation inhibition. During the process of infection, filamentous pathogens that are biotrophic or hemi biotrophic in nature develop specialized structures known as haustoria at the interface where the host and pathogen interact. Pathogenic microorganisms employ small RNA molecules (sRNAs) as a means to enhance the progression of diseases through the selective regulation of host genes. At now, filamentous pathogens do not possess HEN1 orthologs, and it is presumed that the small RNAs (sRNAs) produced by these pathogens are devoid of methylation, as indicated by previous research [35].

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2.3 Regulation of immune receptors by microRNAs

Receptors play a crucial role in the plant immune system, where membrane-bound receptor-like kinases or proteins detect foreign molecular signals, while intracellular NLR receptors identify pathogen effectors and initiate effector-triggered immunity (ETI) [6]. The improper expression or stimulation of immune receptors, specifically NLRs, can result in a decrease in overall fitness and the development of harmful autoimmune

responses [26, 27, 28]. Small RNA molecules (sRNAs) have a significant impact on regulating the expression of nucleotide-binding leucine-rich repeat receptors (NLRs) during various stages of development and in the absence of pathogenic infection. The NLR genes in different plant species are frequently targeted by miRNAs. Among these miRNAs, the miR482/2118 superfamily is conserved and known to target NLR genes by binding to the coding sequence of the highly conserved P-loop motif [29]. The decrease in expression levels of these microRNAs (miRNAs) plays a role in the inducible production of nucleotide-binding domain and leucine-rich repeat containing receptors [NLRs] in response to pathogens. The MiR482/2118 gene family is known to induce the synthesis of secondary small interfering RNAs (siRNAs), which possess the capability to downregulate a greater number of nucleotide-binding leucine-rich repeat (NLR) proteins either in trans or in cis [30, 31, 29]. Additional microRNAs (miRNAs) that target NLR genes, such as miR6019 in tobacco and miR9863 in barley, also exhibit their effects through this dual mechanism of action. Nucleotide-binding leucine-rich repeat [NLR] proteins constitute expansive gene families that exhibit substantial variety within plant genomes, so rendering secondary control using small interfering RNA (siRNA) an appealing approach. Three highly prevalent miRNA families, namely miR1507, miR2109, and miR2118, induce the generation of secondary siRNAs from a substantial portion of the 79 siRNA-generating NLR transcripts, specifically 74 out of the total. The hypothesis posited that a limited number of miRNAs may potentially regulate a substantial quantity of NLRs. Nevertheless, the experimental confirmation of a broad influence of miRNAs on the NLR transcriptome via secondary siRNA synthesis is still lacking. Additional research is required in order to ascertain the fundamental regulatory mechanism and functional consequences of secondary small interfering RNAs (siRNAs) on the overall levels of nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) at a global scale [30].

3. Invasive filamentous pathogens exhibit host-induced gene silencing.

RNA silencing is a phenomenon that occurs outside of individual cells, wherein small RNA molecules (sRNAs) are able to traverse cell boundaries and suppress the expression of specific genes. In the realm of plant biology, it has been observed that small interfering RNAs (siRNAs) and microRNAs (miRNAs) possess the ability to migrate towards pathogens via a phenomenon known as host-induced gene silencing (HIGS) [32]. The HIGS [Host-Induced Gene Silencing] technique has demonstrated efficacy in the management of many diseases, encompassing parasitic plants, fungi, and oomycetes. In recent studies, it has been observed that certain small RNA molecules derived from plants had the ability to suppress the expression of pathogen genes during the course of natural infection, hence serving as an inherent defense mechanism [7, 8, 9].

The silencing of virulence-related genes in the fungal pathogen *V. dahliae* has been observed through the action of two plant miRNAs, namely miR159 and miR166 [9]. The expression of these microRNAs was upregulated upon infection and exhibited sequence complementarity to genes in V. dahliae that encode a cysteine protease (Clp-1) dependent on calcium ions and a hydroxylase (HiC-15) involved in the synthesis of isotrichodermin C-15 [33, 9]. The presence and availability of these miRNAs for gene silencing were observed in fungal cells. The elevated levels of these microRNAs are associated with their mobility and resilience inside the pathogenic organism [34]. Furthermore, it has been observed that secondary small interfering RNAs (siRNAs) can augment the immune response in plants by facilitating host-induced gene silencing. The study demonstrated the efficacy of two tasiRNAs in downregulating target genes within the fungal pathogen *Botrytis cinerea*. This downregulation resulted in a decrease in virulence activities, thereby playing a role in the progression of the disease [35]. A separate investigation revealed that a collection of secondary small interfering RNAs (siRNAs) originating from a specific group of pentatricopeptide repeat (PPR) gene transcripts has the capacity to simultaneously target several genes within the oomycete pathogen *Phytophthora capsici* [8]. The observed result aligns with the high susceptibility phenotype exhibited by the rdr6 mutant of A. Arabidopsis thaliana, a plant species known for its susceptibility to secondary siRNA synthesis, exhibits susceptibility to both diseases. The biological importance of the secondary siRNA route in plant immunity is substantiated by the identification of filamentous pathogens that possess effectors aimed at inhibiting this pathway in plants [10, 11]. The HIGS mechanism necessitates the utilization of small RNA molecules (sRNAs) for the transportation of genetic material from the host organism to the invading pathogen. The mobility of miRNAs and siRNAs may be influenced by variations in their synthesis routes [19]. When the design incorporates identical sequences, it has been observed that tasiRNA-based silencing exhibits a wider spread compared to miRNA-based silence. This observation suggests that secondary siRNAs might possess more mobility and resilience in non-cell-autonomous silencing. This study focuses on the analysis of apoplastic small RNAs (sRNAs) in *Arabidopsis* plants [35]. Arabidopsis thaliana exhibited a notable extracellular aggregation of secondary small interfering RNAs (siRNAs), encompassing TAS-, NLR-, and PPR-siRNAs, hence implying their targeted secretion by plant cells

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[36]. Several microRNAs (miRNAs) exhibit limited mobility, and extracellular vesicles have been found to contain tasiRNAs and PPR-siRNAs as well [7, 8]. Pentatricopeptide repeat gene-derived secondary small interfering RNAs (siRNAs) represent a prominent contributor to the generation of secondary siRNAs in eudicotyledonous plants. The PPR gene family in plants is characterized by its substantial size, consisting of more than 400 members. These proteins possess nucleotide-binding capabilities that are specific to particular sequences. They serve as molecular adaptors, guiding RNA processing complexes to specific target transcripts within mitochondria and chloroplasts. The synthesis of PPR-siRNA can be initiated by directly targeting miRNAs or by the involvement of noncoding TAS genes. The intricate nature of the miRNA-(TAS)-PPR transcripts-siRNA pathways may offer several sites of regulation to finely adjust the levels of PPR-siRNAs, which is a process that is well conserved in dicots. This suggests that these siRNAs have a functional significance, as indicated by previous studies [37, 8, 38].

3.1 Filamentous pathogens engender small RNA molecules.

Eukaryotes, especially fungal and oomycete diseases, exhibit extensive production of small RNAs. The results of target prediction suggest that small non-coding RNAs (sRNAs) derived from these diseases have the capacity to modulate the expression of both endogenous genes inside the pathogens themselves, as well as possible targets within the host organism during the course of infection. Nevertheless, the RNA interference [RNAi] pathway has only been extensively examined in a limited number of model animals, and there has been a scarcity of experimental investigations into the synthesis and functional properties of small RNAs (sRNAs). The reason for this is the presence of technical obstacles in creating mutants within these organisms, which additionally employ a range of sRNA biosynthetic pathways [39]. Phytopathogenic fungus species possess a varied RNA interference (RNAi) pathway consisting of RNA-dependent RNA polymerases (RDRs), Dicer-like proteins (DCLs), and Argonaute proteins (AGOs). The fungal species mentioned in the study demonstrate a wide range of variation in terms of their ecological roles, physical characteristics, and ways of living. This diversity has resulted in the loss of certain genes and the development of new mechanisms for the production of small RNA molecules [40, 41]. For instance, certain fungal species, including *Ustilago maydis*, which is a biotrophic pathogen responsible for causing maize smut disease, lack the essential constituents of the RNA interference (RNAi) machinery [42, 43]. The fungal kingdom has a significant range of variation in terms of ecology, morphology, and lifestyle. There is a proposition that a patrimonial RNA interference (RNAi) pathway, consisting of RNA-dependent RNA polymerases (RDRs), Dicer-like proteins (DCLs), and Argonaute proteins (AGOs), has seen many modifications. These adaptations have resulted in the loss of genes and the emergence of new mechanisms for small RNA (sRNA) synthesis in different fungus species. The pathogen responsible for rice blast, a devastating disease in rice plants, is *Magnaporthe oryzae*, an ascomycete fungus that exhibits a hemibiotrophic lifestyle. The initial experimental evidence for gene silencing mediated by small RNAs (sRNAs) was the utilization of a hairpin RNA derived from the green fluorescent protein (GFP). This hairpin RNA induced the production of small interfering RNAs (siRNAs) measuring 19-23 nucleotides in length, ultimately resulting in the suppression of GFP expression. The examination of various mutations in the core components of RNA interference (RNAi) has yielded a thorough analysis, which has shown the significant involvement of small RNAs (sRNAs) in the regulation of conidia formation and the subsequent reduction in virulence activity specifically in rice [44, 45]. Botrytis cinerea, classified as a necrotrophic pathogen, is responsible for inducing gray mold infections in a wide range of plant species, surpassing 1,400 in number [46]. Research has been conducted to investigate the evolutionary dynamics of small interfering RNA (siRNA)-generating sequences in the organism. The examination of *B.cinerea* isolates and their corresponding target genes across different host plants will yield valuable knowledge regarding the preservation of this virulence mechanism. The growth of Bcdcl1 or Bcdcl2 single mutants was shown to be diminished, and their sporulation was delayed. Furthermore, the Bcdcl1 Bcdcl2 double mutant had a more pronounced developmental deficiency. These findings indicate that small RNAs (sRNAs) also have a role in regulating endogenous genes in the organism *B. cinerea* [47]. *Verticillium dahliae* is classified as a hemibiotrophic pathogen, responsible for inducing *Verticillium* wilt, a very destructive disease that has inflicted severe damage on numerous commercially significant crops. The genome of *Verticillium dahliae* contains three RNA-dependent RNA polymerases (RDRs), two Argonaute proteins (AGOs), a canonical Dicer-like protein [VdDCL1], and an atypical Dicer-like protein (VdDCL2) that lacks one of the two RNase III domains [66]. The mutants of Vddcl1, Vddcl2, Vdago1, and Vdago2 exhibited impairments in hyphal growth and spore development, suggesting a significant involvement of the RNA interference (RNAi) pathway in the developmental processes. *Verticillium dahliae*, a fungal pathogen, is capable of generating small RNA molecules (sRNAs) that range in size from 18 to 25 nucleotides [nt] without displaying any specific size

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preference. The functional characterization of VdmilR1, a 21-nucleotide milRNA with high expression, has been conducted. The precursor transcript of VdmilR1 is anticipated to produce a characteristic stem-loop structure, akin to plant miRNAs. VdmilR1 is designed to specifically target a putative protein-coding gene, VdHy1, in order to induce transcriptional gene silencing (TGS), which is correlated with elevated levels of histone H3K9 methylation. The VdHy1 deletion mutant exhibited decreased hyphal development and melanin formation in the presence of media, along with a significant decrease in virulence when infecting cotton plants. The regulatory mechanisms governing the potential derepression of VdHy1 and subsequent disease enhancement after infection by VdmilR1 remain uncertain. *Fusarium graminearum* is responsible for the development of *Fusarium* head blight (FHB) in wheat, barley, and other cereal crops, leading to significant reductions in crop productivity and contamination of the grain with mycotoxins. The examination of small ribonucleic acids (sRNAs) within mycelia revealed a size range of 17-32 nucleotides [nt], with the most abundant sequences observed at 27-28 nt. Nevertheless, it was shown that the perithecium had a distinct sRNA profile and size distribution, indicating the production of separate sRNA populations throughout various stages of development [28]. *Puccinia striiformis f.sp. tritici* is a fungal pathogen that affects various cereal crops, including wheat and barley. This is a biotrophic pathogenic fungus that induces stripe rust disease in wheat plants. The conventional RNA interference (RNAi) pathway comprises several key enzymes, namely one Dicer-like [DCL] enzyme, one Argonaute [AGO] protein, and two RNA-dependent RNA polymerases (RDRs). However, the precise role of these enzymes in the process of gene silence has not been thoroughly investigated. The complementary small RNA (sRNA) profiles of many phytopathogenic fungi have been examined, although the mechanisms behind the production and role of the RNA interference (RNAi) pathway and the predicted sRNAs have not been fully elucidated [50]. *Phytophthora* species, which are filamentous eukaryotic microorganisms, exert a profound impact as plant diseases, leading to substantial economic losses in the fields of agriculture and forestry. Several well researched species are *P. infestans*, responsible for inducing late blight in potatoes and tomatoes; *P. sojae*, accountable for root and stem rot in soybeans; and *Phytophthora ramorum*, the causative agent of rapid oak death [51]. The availability of a vast collection of genome sequences pertaining to Phytophthora and its associated oomycetes provides an invaluable resource for conducting thorough investigations of small RNA molecules (sRNAs) within these organisms. The existence of active RNA interference (RNAi) is substantiated by the widely recognized target gene knockdown method, which has been utilized to investigate gene functionalities in several Phytophthora species by the implementation of hairpin or antisense RNA constructs. The *Phytophthora* species are known to possess a set of fundamental RNA interference (RNAi) core enzymes, which typically consist of one RNA-dependent RNA polymerase (RDR), two Dicer-like proteins (DCLs), and a variable quantity of Argonaute proteins (AGOs). The presence of two distinct DCLs in each Phytophthora species is evident, as indicated by the formation of two well supported clusters. This observation aligns with the notion that there is an accumulation of two primary classes of small RNAs (sRNAs) in these species, with one class mostly consisting of 21 nucleotides and the other class primarily composed of 25 or 26 nucleotides [52]. Phytophthora species possess a substantial repertoire of cytoplasmic effectors, with a tendency for the genes encoding these effectors to be situated in genomic regions that are rich in repetitive sequences [53]. The process of RNA silencing can have an impact on the expression of neighbouring effector genes by facilitating the establishment of heterochromatin [54]. Small RNA molecules (sRNAs) have the potential to modulate the production of effector molecules, hence influencing their interaction with the host organism. The effective gene silencing in *Phytophthora parasitica* is primarily related with 25/26-nt small RNAs (sRNAs), rather than 21-nt sRNAs [55, 56]. Although Phytophthora species possess a conserved RNA interference (RNAi) pathway, only a single conserved small RNA (sRNA), namely miR8788, has been discovered in *P. infestans*, *P. sojae*, and *P. ramorum*. The microRNA miR8788 triggers the cleavage of its target gene, AAAP, which is responsible for encoding an amino acid/auxin permease. A significant number of small non-coding RNAs (sRNAs) produced from transfer RNA (tRNA), have been identified in *P. sojae*. These sRNAs play a role in the regulation of gene expression by facilitating the targeted destruction of certain RNA molecules through sequence-specific mechanisms. Nevertheless, the biological importance of the control of targets by miR8788 and tRNA-derived small RNAs has yet to be proven (57, 58, 59). Small RNAs, also known as sRNAs, has the ability to exhibit functionality beyond the confines of their originating cells [60]. In the realm of plant biology, it has been observed that both microRNAs (miRNAs) and small interfering RNAs (siRNAs) have the capability to traverse short distances within a plant as well as travel over vast distances through the utilization of plasmodesmata and phloem [61]. In the context of antiviral immunity, it has been observed that small interfering RNAs (siRNAs) had the ability to migrate prior to the onset of infection, thereby initiating the process of viral gene silencing.

4.The phenomenon of small RNA transmission between hosts and pathogens

The presence of ribonucleic acids (RNAs) has been observed in cells that are not infected [35]. The potential of sRNAs to induce plant defense against nonviral diseases through their systemic signalling properties has yet to be experimentally evaluated. In contrast to small interfering RNAs (siRNAs), microRNAs (miRNAs) are characterized by their relatively limited mobility and a close association between their sites of synthesis and their sites of action (4,100). Nevertheless, previous studies have documented the occurrence of local, systemic, and trans-species transfer of certain plant miRNAs. The relationship between SRNA mobility and abundance does not exhibit a direct correlation, indicating the presence of a sorting mechanism that is impacted by factors such as biosynthesis, sequence, and structure of sRNA molecules, as well as their interaction with RNA-binding proteins. Recent studies have brought attention to the phenomenon of trans-species gene silencing, in which small RNA molecules originating from one organism are able to quiet specific target genes in a different organism. The precise mechanisms involved in the migration of small RNA molecules (sRNA), which is a crucial process in the silencing of genes across different animals, are not yet fully understood. The primary objective of this study is to examine and analyze the probable methods by which plant small RNAs (sRNAs) may be transported to invading pathogens [1, 47].

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5.Conclusion

The domain of research pertaining to small RNA molecules (sRNAs) involved in interactions between plants and pathogens/parasites has experienced significant advancements, as evidenced by the documentation of sRNA profiles for numerous pathogens and plants. Several small RNAs (sRNAs) have been subjected to functional characterization due to their involvement in the development of diseases. There is a growing body of evidence indicating that small RNA (sRNA)-mediated gene silencing can potentially take place across different species, representing a noteworthy expansion of the recognized role of sRNAs as mobile regulators and signaling molecules. Gaining a comprehensive understanding of the mechanisms underlying the operation of the Host-Induced Gene Silencing (HIGS) technique is a crucial milestone in the successful integration of small RNA (sRNA)-based defense strategies for disease control [62]. Nevertheless, numerous obstacles remain to be addressed in the field of trans-species gene silencing. The efficacy of silencing is not just determined by sequence complementarity. The impact on the target site structure is primarily influenced by the target site structure itself, rather than sequence complementarity. The optimization of target prediction and the strong confirmation of real targets by genetic and biochemical methodologies are of utmost importance. The confirmation of small RNAs (sRNAs) being loaded into the AGO complexes of the host organism, along with the genetic examination of mutations involving the core enzymes of RNA interference (RNAi), would provide significant evidence in favor of gene silence facilitated by foreign sRNAs through the exploitation of the host's intrinsic mechanism [16]. The stability of small RNA (sRNA) is greatly influenced by its structural characteristics. In plants, the presence of HEN1-mediated 2′-O-methyl modification on the 3′ terminal nucleotide has been found to have a major impact on sRNA stability [63]. Non-methylated small ribonucleic acids (sRNAs) exhibit a lack of stability and are susceptible to swift destruction mediated by nucleases. The investigation of putative strategies employed by pathogens to increase the stability of small RNA molecules during intercellular transport and facilitate their efficient loading onto plant Argonaute proteins is a subject of academic interest. The investigation of sRNA trafficking is a significant field of research, wherein the involvement of EV-independent pathways is explored to understand their role in sRNA trafficking. Prior research indicates that the transit of miRNA can exhibit directionality across particular interfaces between cells. Understanding the mechanisms responsible for sorting and determining selectivity is crucial for small RNAs (sRNAs) that are transported by extracellular vesicles (Evs) [64]. The coevolutionary arms race is a fundamental concept in the field of host-pathogen interaction, characterized by the ongoing reciprocal adaptation of pathogens and their hosts. This dynamic process is reflected in the patterns of diversifying evolution observed in pathogen effectors and NLR receptors. If particular small RNAs (sRNAs) are employed to suppress specific targets in the organisms involved in fighting, it is anticipated that evolutionary characteristics indicative of antagonistic interactions will be detected in both the sequences responsible for creating sRNAs and the genes being targeted. The examination of the evolutionary dynamics of the many elements implicated in the process of trans-species gene silencing will yield crucial understanding regarding the function of small RNAs (sRNAs) in the interactions between hosts and pathogens.

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1.The conclusion of the resistance and pathogenicity in relation to the different types of RNAs.
2.Link and summarize the important findings of the previous studies in a clear and so concise way for the reader.
3.Briefly mention the hints for further studies in light of what you wrote.
4.The conclusion mustn't include a reference, I guess, *i.e*. you just summarize what you mentioned earlier, perhaps. Accordingly, you have either omit references 62 – 64 **OR** mention their contents in the text before concluding.

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