

Tiny Protector's: Exploring the Antiviral Functions of Plants miRNAs

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Abstract- MiRNAs, which are small RNA molecules that do not code for proteins, are essential for regulating gene expression after transcription in plants. Recently, studies have shown that miRNAs also act as antiviral agents in plants by targeting viral RNA and inhibiting viral replication. Several miRNAs have been identified that can target different viral genes and limit viral infection in plants. The role of microRNAs (miRNAs) as antiviral agents in plants. It highlights the importance of understanding the mechanisms by which miRNAs target viral RNA and limit viral infection, as well as the strategies employed by plant viruses to evade miRNA-mediated antiviral defense. The article discusses the potential applications of miRNAs in developing new strategies for controlling viral diseases in crops. It emphasizes that miRNAs not only act as direct antiviral agents by targeting viral RNA but also regulate the expression of genes involved in plant defense responses. Additionally, miRNAs can serve as diagnostic tools for viral infections and targets for the development of antiviral strategies. The article concludes by emphasizing the need for further research to fully understand the mechanisms underlying miRNA-mediated antiviral defense in plants and to develop effective strategies for controlling viral infections in crops.

Keywords: Plant miRNAs, Antiviral mechanisms, Plant immunity, viral suppression, virus resistance, Gene regulation, Host defence response, miRNA expression profiling.

I. INTRODUCTION

Plants are constantly under threat from various pathogens, including viruses that cause significant damage to agricultural crops, resulting in substantial economic losses worldwide. Plant viruses have evolved various mechanisms to evade host immune responses and successfully infect their host plants [1]. In recent years, there has been a growing interest in understanding the role of small non-coding RNAs, particularly microRNAs (miRNAs), in the antiviral defense response of plants. miRNAs are endogenous, single-stranded RNAs of approximately 21-24 nucleotides in length that regulate gene expression post-transcriptionally by targeting mRNA for degradation or translational repression. [2] miRNAs play crucial roles in various biological processes such as development, differentiation, and stress responses in plants.

In addition to their endogenous role, recent studies have shown that miRNAs also play a crucial role in the defense response of plants against viral infections [3]. The identification and characterization of miRNAs as antiviral agents in plants have provided new insights into the molecular mechanisms of

plant-virus interactions and may lead to the development of novel strategies for controlling viral diseases in crops [4]. In this review, we will provide an overview of the role of miRNAs as antiviral agents in plants, their mode of action, and the potential applications of this knowledge in developing new strategies for controlling viral diseases in crops. Food security is continuously being challenged due to various virulent crop pathogens.

These pathogens may include fungi, bacteria, viruses, nematodes and other parasites. Plants have developed a mechanism to protect themselves from pathogens by producing small RNAs (sRNAs) that actively contribute to managing immunity against pathogen attacks. Small RNAs, known as siRNAs and miRNAs, are two types of sRNAs that have been categorized based on their size and function. [5] This review article summarizes the recent progress in understanding the roles of miRNAs as antiviral agents in plants. We discuss the mechanisms by which miRNAs target viral RNA and limit viral infection, as well as the strategies employed by plant viruses to evade miRNA-mediated antiviral defense. Additionally, we highlight recent advances in the development of miRNA-based strategies for

controlling viral diseases in crops. Recently, it has been shown that miRNAs also act as antiviral agents in plants by targeting viral RNA and inhibiting viral replication. This review will summarize the current knowledge on miRNAs as antiviral agents in plants, focusing on their biogenesis, mode of action, and regulation, as well as their potential applications in crop protection. [6]

II. ANTIVIRAL PROPERTIES OF MIRNAS IN PLANTS

Plant viruses cause significant damage to crop yields, affecting global food security. Plant cells have evolved an intricate defense mechanism against viral infections, including RNA interference (RNAi). RNAi is a gene silencing mechanism that relies on the production of small RNA molecules, including miRNAs, that target and degrade viral RNA. miRNAs can also act as activators of the plant immune system by regulating the expression of genes involved in defense responses.[7,8,9,10,11].

Studies have identified several miRNAs that are involved in the defense against plant viruses. For example, miR398 has been found to regulate the accumulation of reactive oxygen species (ROS) in response to viral infection. ROS plays a critical role in plant defense against pathogens, and miR398-mediated regulation of ROS levels can help protect plants from viral infections. Similarly, miR156, miR167, miR168, and miR393 have been shown to regulate the expression of genes involved in antiviral defense responses. [12]

In addition to their direct antiviral properties, miRNAs can also act as markers of viral infection in plants. Studies have identified differentially expressed miRNAs in plants infected with viruses, suggesting that miRNAs could be used as diagnostic tools for viral infections. Furthermore, miRNAs can be used as targets for the development of antiviral strategies in plants. For example, the overexpression of miRNAs that target viral genes could potentially provide broad-spectrum resistance against multiple viruses. [13,14,15,16] miRNAs play a crucial role in the defense against viral infections in plants. They can act as direct antiviral agents and also regulate

the expression of genes involved in plant defense responses. Furthermore, miRNAs can serve as diagnostic tools for viral infections and targets for the development of antiviral strategies. Further research is needed to fully understand the mechanisms underlying miRNA-mediated antiviral defense in plants and to develop effective strategies for controlling viral infections in crops. [17,18,19,20,21]

miRNAs and plants defense against viruses

Plants have evolved various defense mechanisms to protect themselves against pathogens and hostile environments. One of the most important strategies used by plants to combat viral infections is siRNA-mediated gene silencing. This mechanism has two main advantages: the defensive signal can spread, and siRNA is transitive. However, siRNA-mediated gene silencing is triggered only after viruses have invaded the host, thus infected cells are unable to warn non-infected cells until the initial attack by viruses.

Therefore, siRNA-mediated gene silencing alone may not be enough to resist invading viruses, and a proactive mechanism is necessary. Endogenous miRNAs, some of which exist within a cell prior to viral invasion, can serve as advance preparation to counteract or evade the invading virus. Plant miRNAs have evolved to optimize cleavage efficiency rather than maximize complementarity to their targets. Two main modes have been suggested for the roles of miRNAs in an antiviral defense response in plants: a direct mode through targeting viral RNAs, and an indirect mode through triggering the biogenesis of siRNA responsible for the antiviral response. Endogenous miRNAs have been shown to play an important role in the suppression of invading viruses in mammals. In plants, miRNA-mediated post-transcriptional regulation is involved in plant defensive responses against viral infections. [22]

III. MECHANISM OF MIRNAS-MEDIATED ANTIVIRAL DEFENSE

During a viral infection, plants induce a complex defense response that involves various regulatory pathways, including RNA silencing, hormone

signaling, and transcriptional reprogramming. Among these pathways, RNA silencing plays a central role in the plant's antiviral response. RNA silencing is a process by which small RNA molecules, including miRNAs and siRNAs, bind to target RNA molecules, resulting in their degradation or translational repression. [23]

miRNAs can regulate gene expression by base-pairing with target mRNAs and inducing their degradation or translational repression. In the context of viral infection, miRNAs can directly target viral RNA, thereby inhibiting viral replication and spread. For example, miR162 in tomato and Arabidopsis targets the viral RNA-dependent RNA polymerase gene of Tomato spotted wilt virus and Turnip crinkle virus, respectively, thereby inhibiting viral replication. Similarly, miR398 in Arabidopsis targets the mRNA encoding the silencing suppressor protein P38 of Turnip crinkle virus, resulting in decreased viral accumulation. [24]

In addition to direct targeting of viral RNA, miRNAs can also indirectly regulate antiviral defense through the production of siRNA molecules. siRNAs are generated by the RNA-induced silencing complex (RISC) and act as guides to target viral RNA for degradation. miRNAs can induce the biogenesis of siRNAs by targeting mRNAs encoding proteins involved in RNA silencing. For example, miR168 in Arabidopsis targets AGO1, a key component of RISC, resulting in decreased AGO1 protein levels and increased accumulation of siRNAs, which in turn inhibits viral replication. [25,26,27]

Furthermore, miRNAs can also regulate the expression of genes involved in other plant defense pathways, including hormone signaling and transcriptional reprogramming. For example, miR398 in Arabidopsis regulates the accumulation of reactive oxygen species (ROS), which play an important role in plant defense against viral infections. miR398-mediated regulation of ROS levels can help protect plants from viral infections by activating the plant immune system. [28,29,30]

miRNAs Regulatory Role in Plants

MicroRNAs (miRNAs) play crucial roles in gene regulation in plants. They are typically around 20-22 nucleotides in length and are transcribed from endogenous genes by RNA polymerase II [31] Once transcribed, miRNAs are processed by a series of enzymatic reactions to form a mature miRNA, which is incorporated into an RNA-induced silencing complex (RISC). [32] The mature miRNA guides the RISC to its target mRNA through complementary base pairing, resulting in either cleavage of the target mRNA or translational repression. In this way, miRNAs can regulate the expression of a wide range of genes in plants, including those involved in developmental processes, stress responses, and disease resistance. [33]

MiRNA biogenesis begins with the transcription of primary miRNA (pri-miRNA) by RNA polymerase II [34]. The pri-miRNA is then processed into a precursor miRNA (pre-miRNA) by the enzyme Dicer-like 1 (DCL1), which recognizes and cleaves the pri-miRNA at specific sites to generate a double-stranded RNA molecule. [35] The double-stranded RNA is then further processed by the enzyme Hua Enhancer 1 (HEN1), which adds a methyl group to the 3' end of the mature miRNA strand, stabilizing it and preventing degradation by cellular nucleases [36] The mature miRNA is then incorporated into the RISC, which is composed of multiple proteins including AGO1, a protein that plays a central role in miRNA-mediated gene regulation [37].

MiRNAs have been shown to regulate a wide range of developmental processes in plants, including leaf and flower development, root development, and stem cell maintenance. [38] For example, miR166 is involved in the regulation of leaf polarity, while miR319 regulates leaf shape and size. In addition to their roles in development, miRNAs also play important roles in plant responses to biotic and abiotic stress. For example, miR398 is involved in the regulation of copper homeostasis, while miR398b/c are involved in regulating plant responses to oxidative stress. MiRNAs also play important roles in plant-microbe interactions, including the regulation of plant immune responses to pathogens [39] In conclusion, miRNAs are important regulators of gene expression in plants, with roles in development,

stress responses, and disease resistance. The precise mechanisms by which miRNAs regulate gene expression are complex and are still being elucidated, but it is clear that they play a crucial role in ensuring proper gene expression and function in plants. As our understanding of miRNA function in plants continues to expand, it is likely that they will be increasingly utilized in the development of novel strategies for crop improvement and protection.

IV. HARMFUL EFFECTS CAUSED BY VIRUSES IN PLANTS

Plant viruses can reduce a plant's yield in several ways. Firstly, viruses can cause stunted growth and deformation of the plant, resulting in smaller fruits and reduced yield overall.[40]. This is because the virus can interfere with the plant's normal growth and development processes. Secondly, viruses can cause discoloration and spotting of leaves, which can reduce the plant's ability to carry out photosynthesis[41]. Photosynthesis is the process by which plants produce energy, and if this process is impaired, the plant may not have enough energy to produce a good yield. Thirdly, viruses can weaken the plant's immune system, making it more vulnerable to other diseases and pests. This can further reduce the plant's yield and quality[42].

Finally, viruses can reduce the amount of nutrients that the plant is able to absorb from the soil, leading to nutrient deficiencies that can affect the plant's growth and yield. Overall, plant viruses can have a significant impact on crop yield, and effective management strategies are necessary to reduce their spread and impact. Plant viruses can have several harmful effects on plants, including Reduced yield: Plant viruses can cause stunted growth, deformation, and discoloration of leaves, which can reduce photosynthesis and limit the plant's ability to produce energy. This can lead to smaller fruits and a decreased overall yield. Lowered quality: Viruses can also reduce the quality of the fruits and vegetables produced by infected plants. For example, viruses can cause discoloration, spotting, or even necrosis (death) of the fruit, making it less marketable and less desirable to consumers. Spread to other plants: Once a plant is infected with a virus, it can easily

spread the virus to other plants in the field. This can lead to the rapid spread of the virus and increased damage to crops. Reduced lifespan: Viruses can also shorten the lifespan of plants, reducing their ability to produce for multiple seasons and decreasing overall crop yields over time. Overall, plant viruses can have a significant impact on the agricultural industry, causing economic losses for farmers and reducing the availability and quality of food for consumers. It is essential to take preventative measures, such as crop rotation, use of virus-free planting material, and proper sanitation, to minimize the spread and impact of plant viruses.

V. DISEASE CAUSED BY VIRUSES IN PLANTS

Plant viruses can cause a wide range of diseases in plants, which can affect their growth, yield, and overall health. These diseases can be characterized by symptoms such as yellowing or discoloration of leaves, stunted growth, deformation or distortion of plant parts, and reduced fruit or seed production.[43] Plant viruses can also cause other physiological changes in the plant, such as alterations in the plant's metabolism or the way it uses nutrients [44]. There are many different types of plant viruses, and each can cause unique symptoms and diseases in specific plants or plant families. Some examples of plant viruses and the diseases they cause include:

Tobacco mosaic virus (TMV): Causes mosaic patterns and necrotic spots on leaves, stunted growth, and reduced yield in tobacco and other solanaceous plants: Affects tobacco plants and causes mosaic-like patterns on the leaves. Tobacco mosaic virus (TMV) is a plant virus that belongs to the genus Tobamovirus. It is named so because it majorly infects tobacco plants, potatoes, tomatoes and other members of the Solanaceae family. The infection creates a mosaic like pattern, mottling and discoloration of the leaves. Let us look at the discovery, structure and lifecycle of the virus. The virus gets inside the plant host cells via vectors such as aphids, flies and other insects.

After entering the host cell the virus starts to multiply and spreads to the nearby cells via

plasmodesmata. The virus can be transmitted to other healthy plants by coming in direct contact with the infected plants. For replication and multiplication inside the host cell, the viral particle releases its genomic material. The ssRNA then translates multiple mRNAs that can replicate the viral genome and the capsid proteins. All these proteins assemble to form a newly synthesised cell of tobacco mosaic virus that is ready to infect another cell. [45]

Tomato yellow leaf curl virus (TYLCV): Affects tomato plants and causes yellowing and curling of leaves, stunted growth, and reduced yield. Tomato yellow leaf curl virus (TYLCV) is a plant virus that infects tomato plants and other members of the family Solanaceae, such as peppers and eggplants. It is transmitted by the whitefly, *Bemisia tabaci*, which feeds on the sap of infected plants and spreads the virus to healthy ones.

TYLCV causes severe symptoms in infected plants, including yellowing and curling of leaves, stunted growth, and reduced fruit yield and quality. The virus can also cause the death of young plants is a major threat to tomato production in many parts of the world, particularly in the Middle East, North Africa, and the Mediterranean region. Control measures for TYLC include the use of resistant tomato varieties, insecticides to control whitefly populations, and good cultural practices to minimize virus spread.

Potato virus Y (PVY) is a plant virus that primarily affects potatoes (*Solanum tuberosum*), although it can also infect other plants in the Solanaceae family, such as tomatoes, peppers, and tobacco. PVY is one of the most economically significant viral pathogens in potato production worldwide. PVY belongs to the genus *Potyvirus* and the *Potyviridae* family. It is a single-stranded RNA virus that is transmitted by aphids, particularly species from the genera *Myzus* and *Aphis*. The virus can also spread through infected seed tubers, mechanical means (e.g., contaminated tools), and grafting. Symptoms of PVY infection in potato plants can vary depending on the strain of the virus and the potato cultivar.

Common symptoms include mosaic patterns on the leaves, leaf curling, stunted growth, and necrotic

(brown or black) streaks on the tubers. In some cases, the infection may not show any visible symptoms, making it difficult to detect. PVY can cause significant yield losses in potato crops. The virus affects both the quality and quantity of the harvested tubers. Infected plants may produce smaller and misshapen tubers, reducing market value. Additionally, PVY-infected tubers are more susceptible to secondary bacterial and fungal infections.

Controlling PVY requires an integrated approach. Some common strategies include: Using certified virus-free seed tubers: Planting certified seed tubers that are tested and certified as free from PVY reduces the risk of introducing the virus into the crop. Aphid control: Implementing aphid control measures, such as insecticide sprays or the use of physical barriers like insect-proof nets, can help reduce the transmission of PVY.

Rogueing: Removing and destroying infected plants during the growing season can help prevent the spread of PVY to healthy plants. Crop rotation: Practicing crop rotation by avoiding planting potatoes or other susceptible crops in the same field for several years can help reduce the virus population in the soil. Virus-resistant potato cultivars: Planting potato varieties that possess genetic resistance to PVY can provide an effective means of control. It is essential for farmers and growers to follow recommended management practices and consult with local agricultural extension services or plant pathologists for guidance on specific control measures for PVY in their region. [46]

Cucumber mosaic virus (CMV): Causes mottling, distortion, and stunting of leaves, and reduced yield in cucurbits and other crops. Affects cucumbers, tomatoes, and peppers, causing mosaic-like patterns on leaves and stunted growth. CMV is primarily transmitted through various means, including insect vectors such as aphids, mechanical transmission through contaminated tools or human activities, and seed transmission. Once a plant is infected, CMV can spread systemically, meaning it moves throughout the entire plant, including the leaves, stems, and fruits. Symptoms of CMV infection can vary

depending on the host plant and environmental conditions. However, some common symptoms include mosaic patterns on leaves (irregular patterns of light and dark green), leaf distortion, stunting, yellowing, and reduced fruit yield. In severe cases, CMV infection can lead to plant death. The impact of CMV on agricultural crops can be significant, causing economic losses due to reduced crop quality and yield. Additionally, CMV infection can weaken plants, making them more susceptible to other diseases or environmental stresses.

Management of CMV primarily involves preventive measures such as using virus-free planting material, practicing good sanitation, controlling insect vectors, and eliminating weed hosts. There are no specific treatments for viral infections in plants, so prevention and control measures are crucial to minimizing the spread and impact of CMV. It's worth noting that this information is based on the knowledge available up until September 2021, and there may have been further developments or research on CMV since then. **Bean yellow mosaic virus:** Affects bean plants and causes yellowing and mottling of leaves, stunted growth, and reduced yield.[47]

Tomato spotted wilt virus (TSWV): Tomato spotted wilt virus (TSWV) is a plant pathogenic virus that infects a variety of plants, including economically important crops like tomato, pepper, and tobacco. It is transmitted by thrips, specifically *Frankliniella occidentalis*. TSWV causes symptoms such as necrotic spots, ringspots, chlorosis, wilting, and stunting in infected plants. The virus employs various strategies to evade the host's immune responses. Control measures include the use of resistant cultivars, integrated pest management, and insecticides. Research aims to understand the virus-host interactions and develop effective control strategies.

TSWV causes necrosis and wilt of leaves, reduced yield, and poor fruit quality in many crops, including tomato, pepper, and lettuce [48]. Overall, plant viruses are a significant threat to agriculture and can cause substantial economic losses for farmers. Effective management strategies, including the use

of virus-free planting material, proper sanitation, and resistant cultivars, can help reduce the spread and impact of plant viruses.

Maize dwarf mosaic virus (MDMV): Affects maize plants and causes yellowing and stunting of the plant, reducing yield. Maize dwarf mosaic virus (MDMV) is a plant virus that infects maize plants, causing symptoms such as stunting, yellowing, and mosaic patterns on the leaves. It is a member of the genus *Potyvirus*, which includes many other plant viruses. MDMV is transmitted by several species of aphids in a non-persistent manner, which means that the virus does not replicate within the insect and can be quickly transmitted to healthy plants by feeding aphids. It can also be transmitted mechanically, for example through contaminated tools or plant sap.

The virus can have a significant impact on maize crop yields, particularly in areas with high aphid populations. Management strategies include the use of resistant maize varieties, control of aphid populations, and strict sanitation practices to prevent mechanical transmission. These are just a few examples of the many plant diseases caused by viruses. Effective management strategies, such as the use of virus-free planting material and proper sanitation, can help reduce the impact of these diseases on crops [49]

miRNAs in plant-virus interactions

Plant viruses are one of the major threats to global food security, causing significant economic losses every year. Plants have developed complex defense mechanisms to protect themselves against viral infections. Among these mechanisms, the role of miRNAs has gained increasing attention in recent years. miRNAs are small non-coding RNAs that regulate gene expression at the post-transcriptional level. In this section, we will discuss the role of miRNAs in plant-virus interactions[50].

A. miRNAs as targets of viral suppressors

Plant viruses have evolved various strategies to counteract host antiviral defense mechanisms, including targeting miRNAs. Viral suppressors of RNA silencing (VSRs) are proteins encoded by plant viruses that inhibit the RNA silencing pathway, which

includes miRNA-mediated gene regulation. VSRs can directly target and degrade host miRNAs, or interfere with their biogenesis or function. For example, the Turnip crinkle virus P38 protein suppresses miRNA-mediated gene silencing by directly binding to host miRNAs and sequestering them from their targets. The Potato virus X P25 protein inhibits miRNA biogenesis by binding to the host Dicer-like 1 (DCL1) protein, which is involved in miRNA processing. The Tomato spotted wilt virus NSs protein targets the host RNA-dependent RNA polymerase 6 (RDR6), which is required for miRNA amplification. These examples demonstrate how VSRs can target miRNAs to facilitate viral infection[51].

B. Regulation of viral gene expression by host miRNAs

In addition to being targeted by VSRs, miRNAs can also regulate viral gene expression. Plant miRNAs can recognize and bind to viral RNA, leading to degradation or translational repression of the viral genome. For example, the Arabidopsis miRNA miR168 targets the messenger RNA of the AGO1 protein, a key component of the RNA-induced silencing complex (RISC), resulting in the degradation of the AGO1 mRNA and the subsequent accumulation of siRNAs that target viral RNA for degradation. Similarly, the miRNA miR159 in Arabidopsis targets the messenger RNA of the F-box protein, which is required for the replication of the Cucumber mosaic virus. The repression of F-box expression by miR159 leads to reduced viral replication[52].

miRNAs	Defensive role of plant species	Name of pathogen	Target gene
amiR159	<i>Arabidopsis</i>	TYMV	P69, HC-Pro
miR159a	<i>N. benthamiana</i>	PPV	P1/HC-Pro
miR167b	<i>N. benthamiana</i>	PPV	P1/HC-Pro
miR171a	<i>N. benthamiana</i>	PPV	P1/HC-Pro
amiR171	<i>N. tabacum</i>	CaMV	2b
miR1885	<i>Brassicca napus</i>	TuMV	TIR-NBS-LRR
Pre-miR171a	<i>Arabidopsis</i>	CMV	3'-UTR
Pre-miR159	<i>Arabidopsis</i>	TuMV	P69
miR159a	<i>N. tabacum</i>	PVY	HC-Pro
miR167b	<i>N. tabacum</i>	PVX	TGBp1/p25
miR171a	<i>N. tabacum</i>	PVX	TGBp1/p25
Pre-miR159a	<i>Solanum lycopersicum</i>	CMV	2a, 2b
miR482	<i>Solanum lycopersicum</i>	TCV, CMV, TRV	NBS-LRR
Pre-miR159a	<i>N. benthamiana</i>	WSMoV	Lreplicase gene (Conserved motifs)
miR395	<i>Triticum</i>	WSMV	Conserved region
Pre-miR319a	<i>Vitis vinifera</i>	GFLV	Coat protein (CP)
amiR-AV1-1	<i>Tomato</i>	ToLCNDV	AV1 and AV2
Pre-miR169a	<i>N. benthamiana</i>	CLCuBuV	V2 gene
Pre-miR319a	<i>S. lycopersicum</i>	ToLCV	AV1 and AV2 (coat protein)
Pre-miR319a	<i>N. benthamiana</i>	PVY	CI, NIa, Nib, CP
miR6019/ miR6020	<i>N. tabacum</i>	TMV	TIR-NBS-LRR
Pre-miR171	<i>N. benthamiana</i>	WDV	Conserved region
Pre-miR528	<i>Oryza sativa</i>	RSV, RBSDV	Middle segment, 30 end
Pre-miR159a	<i>N. benthamiana</i>	CBSV, UCBSV	P1, P3, CI, Nib and CP
Pre-miR159a	<i>N. benthamiana</i>	TSWV	N, NSs

C. Role of viral miRNAs in modulating plant antiviral defense

In addition to host miRNAs, some plant viruses encode their own miRNAs, which can modulate host gene expression and suppress antiviral defenses. For example, the Rice black streaked dwarf virus encodes a miRNA that targets the host gene encoding a nucleotide-binding leucine-rich repeat (NLR) protein, which is involved in plant immunity. The repression of NLR expression by the viral miRNA leads to increased viral replication. Similarly, the Cucumber mosaic virus encodes a miRNA that targets the host RNA silencing suppressor protein, leading to enhanced antiviral defense. These examples demonstrate how viral miRNAs can manipulate host gene expression and affect the outcome of plant-virus interactions. In conclusion, miRNAs play a crucial role in plant-virus interactions.

They can be targeted by VSRs to facilitate viral infection, or they can target viral genes to limit viral replication. Furthermore, some viruses encode their own miRNAs to manipulate host gene expression and suppress antiviral defenses. The study of miRNAs in plant-virus interactions is a rapidly evolving field, and further research is needed to fully understand the complex mechanisms involved in this process[53]. miRNA have emerged as key players in plant biotechnology due to their ability to regulate a wide range of biological processes, including defense against viral infections. This paper aims to provide an overview of the current applications of miRNAs in plant biotechnology, with a focus on miRNA-based approaches for engineering viral resistance in plants, the development of miRNA-based antiviral strategies in crop protection, and the potential challenges and future prospects of miRNA-mediated antiviral therapy in plants.[54]

A. miRNA-based approaches for engineering viral resistance in plants:

One of the most promising applications of miRNAs in plant biotechnology is their use in engineering viral resistance in plants. miRNAs can be used to target viral genes and interfere with viral replication, thus providing a new approach to control viral

infections in crops. For example, miRNA-mediated targeting of the conserved regions of the coat protein gene of Papaya ringspot virus (PRSV) has been shown to confer resistance against the virus in transgenic papaya plants (Hsieh et al., 2011). Similarly, the overexpression of miR156 in Arabidopsis has been shown to confer resistance against Cucumber mosaic virus (CMV) by targeting the replication and movement proteins of the virus (Jayasena et al., 2019).[55]

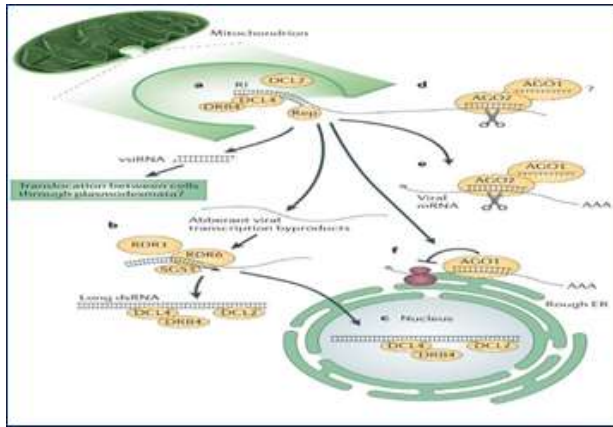
B. Development of miRNA-based antiviral strategies in crop protection:

In addition to engineering viral resistance in plants, miRNAs can also be used to develop novel antiviral strategies in crop protection. One such strategy involves the use of synthetic miRNAs (smiRNAs) to target viral genes and inhibit viral replication. For example, the use of smiRNAs targeting the coat protein gene of Tomato yellow leaf curl virus (TYLCV) has been shown to provide effective protection against the virus in transgenic tomato plants (Ji et al., 2018). Another approach involves the use of miRNA mimics to stimulate plant defense responses against viral infections. For example, the overexpression of miR399 in Arabidopsis has been shown to enhance plant defense against Turnip mosaic virus (TuMV) by inducing the expression of defense-related genes (Sun et al., 2015). [56]

C. Potential challenges and future prospects of miRNA-mediated antiviral therapy in plants:

Despite the promising applications of miRNAs in plant biotechnology, there are several challenges that need to be addressed before miRNA-based antiviral therapy can be widely used in crop protection. These include the development of efficient and specific delivery systems for miRNAs, as well as the potential for off-target effects and unintended consequences of miRNA-mediated gene regulation. However, recent advances in genome editing technologies such as CRISPR/Cas9 may provide new opportunities to overcome these challenges and further enhance the potential of miRNA-mediated antiviral therapy in plants (Ali et al., 2020). Overall, miRNA-based approaches have significant potential for improving crop protection and sustainability in agriculture.[57]

FIGURE



VI. MOLECULAR AND CELLULAR BASES FOR ANTIVIRAL SILENCING

Reverse genetic data have unequivocally established the antiviral nature of RNA silencing through viral double-stranded RNA (dsRNA) processing by dicer-like 4 (DCL4) and DCL2 and targeting of viral genomes or transcripts by virus-derived small interfering (vsRNA)-loaded ARGONAUTE 1 (AGO1) and AGO2. However, the molecular and cellular mechanisms underlying these two steps remain obscure. The figure depicts non-mutually exclusive, alternative possibilities that are consistent with the current data. a-c | Possible modes of DCL action.

Dicing might occur directly on viral dsRNA formed during replication (part a), linked to RNA-dependent RNA polymerase (RDR) activity in the cytoplasm (the habitat of RNA viruses) (part b) or might occur on viral RNA entering the nucleus from the cytoplasm before or after RDR action (part c). d-f | Possible modes of AGO action. Viral RNA slicing by AGO2 might occur at the site of replication (here, mitochondrial membranes), although whether AGO1 slices viral RNA remains unknown (indicated by the question mark) (part d); viral RNA slicing might occur on viral transcripts that are destined for translation (part e).

Translational repression of viral transcripts on the rough endoplasmic reticulum (ER) might occur through the action of AGO1 (part f). Additionally, vsRNAs might move from cell to cell through the plasmodesmata to immunize surrounding, naive

tissues. DRB4, DOUBLE-STRANDED-RNA-BINDING PROTEIN 4; Rep, replication-associated protein; RI, replication intermediate.

VII. CONCLUSION

This review paper has highlighted the important role of miRNAs in the defense against viral infections in plants. It has been demonstrated that miRNAs can act as direct antiviral agents by targeting viral RNA for degradation and also regulate the expression of genes involved in plant defense responses. Moreover, miRNAs can serve as diagnostic tools for viral infections and targets for the development of antiviral strategies. Plant viruses are a major threat to crop production worldwide.

Plants have evolved sophisticated defense mechanisms to combat viral infections, including the use of small RNAs, particularly miRNAs. This paper has provided an overview of the role of miRNAs in the defense against plant viruses. Firstly, miRNAs can act as direct antiviral agents by targeting viral RNA for degradation. For example, miR168 in Arabidopsis targets AGO1, a key component of the RNA-induced silencing complex (RISC), resulting in decreased viral replication. Secondly, miRNAs can indirectly regulate antiviral defense through the production of siRNA molecules, which act as guides to target viral RNA for degradation.

Thirdly, miRNAs can regulate the expression of genes involved in other plant defense pathways, including hormone signaling and transcriptional reprogramming. Finally, miRNAs can act as markers of viral infection in plants and be used as targets for the development of antiviral strategies. Further research is needed to fully understand the mechanisms underlying miRNA-mediated antiviral defense in plants and to develop effective strategies for controlling viral infections in crops. This will involve the identification of new miRNAs involved in antiviral defense, as well as the development of novel approaches for miRNA delivery and targeted gene editing. miRNAs play a crucial role in the defense against viral infections in plants, and further research is needed to fully harness their potential for the

development of sustainable and effective strategies for controlling viral infections in crops.

B. Significance of miRNA-based antiviral strategies for sustainable agriculture

The development of miRNA-based antiviral strategies has significant potential for sustainable agriculture. Viral infections are a major threat to crop production and can result in significant economic losses. Traditional methods for controlling viral infections, such as the use of pesticides, can be costly and have negative impacts on the environment. Therefore, the development of miRNA-based antiviral strategies could provide a more sustainable and cost-effective approach for controlling viral infections in crops. One potential application of miRNA-based antiviral strategies is the development of genetically modified crops that overexpress miRNAs targeting viral genes.

This approach has been shown to provide broad-spectrum resistance against multiple viruses (Garcia-Ruiz et al., 2015; Mitter et al., 2013). Another potential application is the use of miRNAs as diagnostic tools for viral infections, which can enable early detection and rapid response to outbreaks. In addition to their potential for controlling viral infections in crops, miRNA-based antiviral strategies also have broader implications for sustainable agriculture. By reducing the reliance on pesticides and other harmful chemicals, these strategies can promote more environmentally friendly agricultural practices. Furthermore, by improving crop yields and reducing economic losses due to viral infections, miRNA-based antiviral strategies can contribute to global food security.

Overall, the development of miRNA-based antiviral strategies has significant potential for sustainable agriculture. However, further research is needed to fully understand the mechanisms underlying miRNA-mediated antiviral defense in plants and to develop effective strategies for controlling viral infections in crops.

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