4

PERFORMANCE OF ENGINEERING PLANT VIRUS RESISTANCE: miRNA GENE SILENCING

Elizah Mohamed, Nur Aqilah Zawani Mustafa, Husna Izzati Sani, Mohd Haiqal Abd Aziz

Faculty of Engineering Technology,
Universiti Tun Hussein Onn Malaysia,
Pagoh Higher Education Hub,
KM 1, Jalan Panchor,
84600 Muar, Johor, Malaysia.

Abstract—MicroRNAs (miRNAs) are non-coding RNAs of around 20–24 nucleotides long that fill in as focal controllers of eukaryotic quality articulation by focusing on mRNAs for cleavage or translational suppression. In plants, miRNAs are related to various administrative pathways in development and improvement cycles, and cautious reactions in plant-microbe associations. Recently, significant progress has been made in understanding miRNA-interceded gene silencing and how infections counteract this guard system. Genetic improvement in protection from plant infections is the way to manageable practices. A few new advancements have been actualized in plant antiviral building in the most recent decades. RNA hushing and genome altering are the two significant antiviral systems as the outline for this situation. RNA hushing is an indigenous, safe control eukaryotic system that controls quality articulation by little RNAs (sRNAs). The exhibition of infection opposition is clarified as far as RNA quality quieting. There are two kinds of RNA quieting which are siRNA and miRNA. The two safeguards are marginally exceptional, yet they have a very basic portrayal and capacities. This infection impediment is critical. This infection obstruction is crucial. The gene can adapt to and deal with infections that minimise yield production.

Keywords— *Agricultural biotechnology, Virus resistance, miRNA, Gene adjustment, barriers.*

4.1 Introduction

Since the beginning of the agricultural revolution, farmers have faced a critical and significant challenge due to plant diseases and pests. The global population has increased by more than a quarter in the last two decades, rising from 7.7 billion in 2019 to around 10 billion by 2050. As the world's population continues to grow, one of the most pressing issues is the availability of sufficient food [1]. Pests and diseases further limit global food production growth due to limited land that can be ploughed and water resources. Plant yields are threatened by pests, which cause yield losses of 20–40%, while bacterial and fungal pathogens reduce crop yields by 15% and viruses by 3–7%. The number of deaths caused by viruses is quite low, but viral disease infections can cause serious

problems. Chemical pesticides can be used to control bacterial or fungal pathogens as well as insect pests; however, there is currently no conventional chemical pesticide that directly combats viral diseases. As a result, the major issue of economic losses caused by viral diseases will almost always exceed USD 60–80 billion each year. Viral diseases severely reduced crop yield and quality, threatening global food security. The virus in the agricultural sectors should be concerned because it will slow economic growth. The objectives for achieving sustainability are economic vitality, community health, and the natural environment. Currently, proposals and methods for combating infectious diseases in the field include using pesticides or natural predators to combat vectors, or using physical barriers such as clear mulches and microbe-proof networks [2].

4.1.1 Agricultural Biotechnology

In order to promote the conservation of biodiversity, a sustainable idea needs to be considered to nurture the earth. A reduction in soil loss and unnecessary overuse of fertilizers, pesticides, fungicides, herbicides and agricultural runoff are required to this policy.A demand can be made for biodiversity conservation as a source of traits for the integration of different genetic resources into food plants and animals, but an even stronger case can be made for biodiversity conservation to provide ecological services that will sustain the earth and its inhabitants into the future. The most effective way to encourage biodiversity is to protect natural ecosystems in light of the serious threats to biodiversity from a variety of sources. By sustaining or even increasing yields on established fields, biotechnology crops can help mitigate the spread of agriculture into natural areas. As previously reported, agricultural biotechnology has modified pesticide spraying to significantly reduce greenhouse gas emissions and the environmental effects of insecticides and herbicides. It is expected that gene flow from crop species, including biotechnology-based crops, to and from wild plants will occur. In fact, the effects of this flow, while varying from species to species, do not cause serious harm to biodiversity.

4.1.2 Virus Resistance – RNA Gene Silencing

Because the use of virus-resistant varieties in agricultural production is the most efficient and sustainable way of minimising the damage caused by viral diseases, the current scenario necessitates the development of highly effective and durable virus-resistant crop varieties to avoid ever more serious viral diseases. The key to long-term practices is genetic improvement in plant virus resistance. In the last few decades, several new technologies in plant antiviral engineering have been implemented. As summarized in this case, RNA silencing and genome editing are two important antiviral strategies [3]. RNA silencing resistance in plants is one of the antiviral strategies to be focused on. Short interfering RNA (siRNA) and microRNA are the two types of RNA silencing resistance (miRNA) (see Figure 4.1). RNA silencing is an ancient, conserved eukaryotic mechanism that regulates gene expression via small RNAs (sRNAs). RNA silencing is a mechanism in plants that results in the sequential degradation of cellular and viral RNAs at the post-transcriptional stage [4]. Silencing of RNA is caused by double-stranded RNA (dsRNA), which is formed into 21 to 24 nucleotide (nt) molecules known as small interfering RNA (siRNA) and microRNA (miRNA).

4.1.3 siRNA

Small interfering RNAs are double-stranded RNA molecules that are 21 to 24 nucleotides long and have 2 to 3 nucleotide ridges at the 3-strand terminal. SiRNAs are typically produced by RNase III (Dicer) cleavage of long double-stranded RNAs. This is required for the hydroxylated 3-strand terminal, which is thought to be required for the siRNA-primed replication stage catalysed by RdRps. Because of the foregoing, siRNAs at the 5-strand terminal should be phosphorylated by intrinsic internally kinases to gain access to the RISC complex, but this will not have a negative impact on

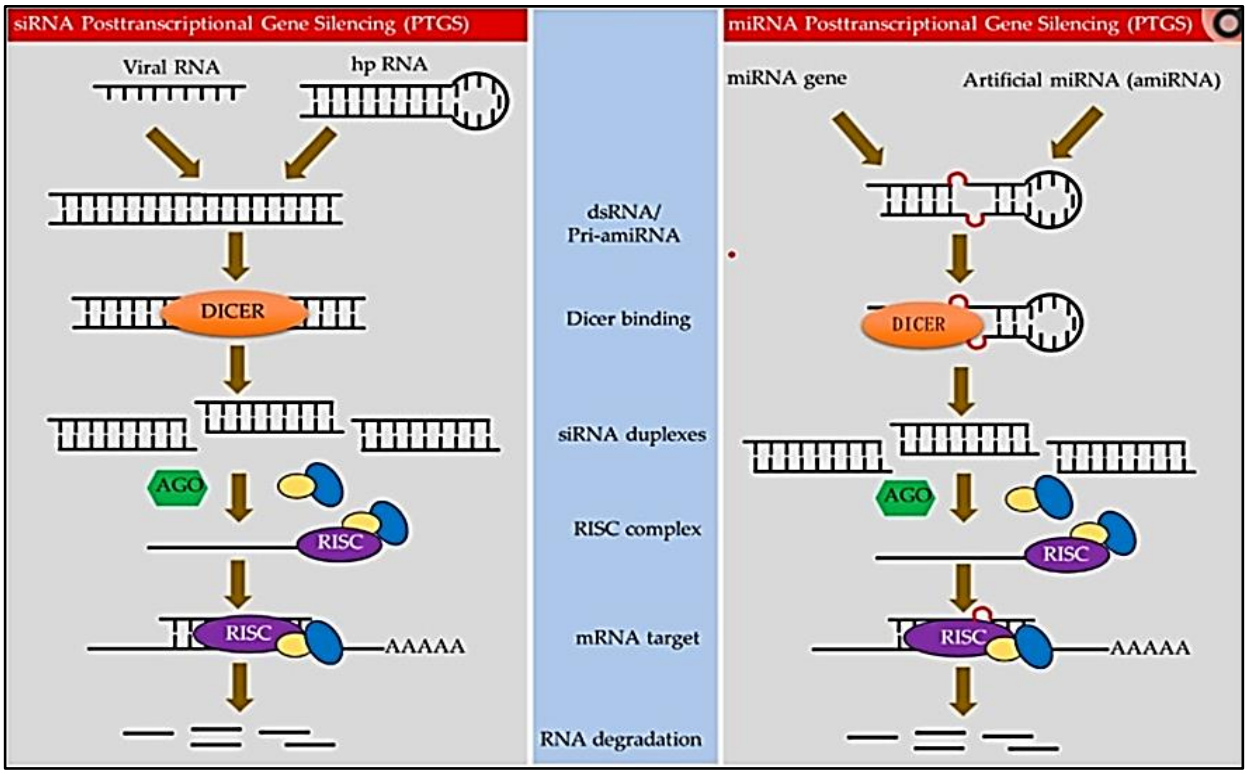

Figure 4.1: Various RNA-mediated gene silencing in perennial plants [3].

RNAi. mediated silencing by anti-priming in the 3-strand terminal hydroxyl group SiRNAs function as RNA guides for trait suppression, but not as foundationfor human and Drosophila RNAi mechanisms. These findings support the theory that each strand of siRNA serves different functions in the RNAi cycle, as does the 3′ hydroxylated end of the antisense strand, which leads to propagation. Endogenous siRNA was found in over 500 wild-type C. Elegans genes. According to the evidence, siRNA is a common molecule among species and a globally conserved molecule.

4.1.4 miRNA

MiRNAs, rather than siRNA, are another type of RNA silencing. Using Dicer-mediated endogenous 70-nt non-coding stem-loop catalysts, 19-25 nucleotide small RNA species known as miRNAs will be formed. Lin-4 and let-7 miRNAs were the first to be discovered in C. Elegans. It may cause mRNA failure in plants while allowing for match confusion. Approximately 2,000 different types of miRNA have been described in plants, animals, and lower organisms up to this point. Although some miRNAs are epigenetic modifiers, others are species-specific or specific to different stages of development. It differs from another term. MiRNAs with well-defined structures, such as lin-4 and let-7, are related to small transient RNAs (stRNAs), whereas miRNAs are small RNAs with unknown functions.

Multi miRNAs have been discovered to play a physiological role in cancer and other infections. Table 4.1 contains a few listings that differentiate between siRNA and miRNA.

4.2 RNAI'S Evolutionary Relevance in Immunologic Responses

RNAi can provide a systemic solution for immunising an organism against invasive nucleic acids by inducing RNAi responses. RNAi is used in plants to achieve virus-induced gene silencing (VIGS). There are numerous known virulent genetic links to RNAi. Many plant virus codes for suppressors of the gene-silencing virus can be found there (VSGS). VSGS functions as a virulence determinant and is thus required in the host to develop an anti-virulence response. As a response to the virulence, the host can alter their PTGS/RNAi pathways to protect against future infections. Only RNAi has the ability to target DNA virus replication in plants. VSGS functions as a virulence determinant and is thus required for the development of anti-virIGS mechanisms not only in plants and nematodes, but also in some species, such as Drosophila-infected viruses, flock house

virus (FHV), and possible silencer suppressor codes (b2).

Table 4.1: The comparison between siRNAs and miRNAs [5].

Resemblances	
siRNA	**miRNA**
The siRNA needs the systems for long double stranded RNAs	The miRNA need processing from stem-loop precursors that are ~70 nt long
An RNase III enzyme is required for processing	Dicer is required
The siRNAs are usually ~22 nt long	The miRNA are also ~22nt long
Disparities	
siRNA	**miRNA**
The siRNA are double stranded structures with 2-nt 3' overhangs that are formed during cleavage by Dicer	The miRNAs are single-stranded structures
The siRNA requires high homology with the mRNA to bind and cleave	The miRNAs can function even with a few mismatched nucleotides
The siRNA mediate target mRNA cleavage by RISC	The miRNA can either block target mRNA translation by binding to it or mediate target mRNA by cleavage by RISC
The siRNAs are usually triggered by transgene incorporation, viral infection, or active transposons	The miRNAs are constitutively expressed cellular RNA moieties with potential roles in development, and cell proliferation and death

4.3 Application of RNAi Silencing

More active resistance mediated by silencing RNA (Figure 4.2-4.3) against RNA viruses is now documented. In contrast to active resistance to RNA viruses, effective resistance to DNA viruses has been rare. Gemini viruses are plant DNA viruses with a single-stranded and circular DNA genome that appear to be resistant to silencing RNA. Fortunately, the gemini viral promoter has the ability to suppress transgene expression when it comes into contact with a homologous gemini virus. Unlike viral plant pathogens, which multiply and reproduce inside diseased plant cells, encounters between other fungal plant pathogens and their respective hosts occur via an extremely specific cell known as haustorium, which is enclosed by an extra haustorial matrix bordered by fungal membranes and plant on either side. This is the platform for signal transmission and nutrient uptake. Close communication between interaction partners, which frequently promotes the absorption of dsRNA or siRNA by host plant cells into the fungal of fungal, has generated silencing-mediated resistance to

RNA. Several trials from previous studies have been conducted to directly inject and administer exogenous dsRNA into insects to express target genes. Reduced production of root knot nematodes, also known as Coleoptera Lepidoptera insects, fed on transgenic plants containing RNAi works against these pests' target genes. The application of dsRNA or siRNA in these animals is accomplished by sucking on plant material, which is accompanied by resorption in the (mid) gut region, which may render this process stable and cost-effective for insects due to RNA silencing-mediated resistance.

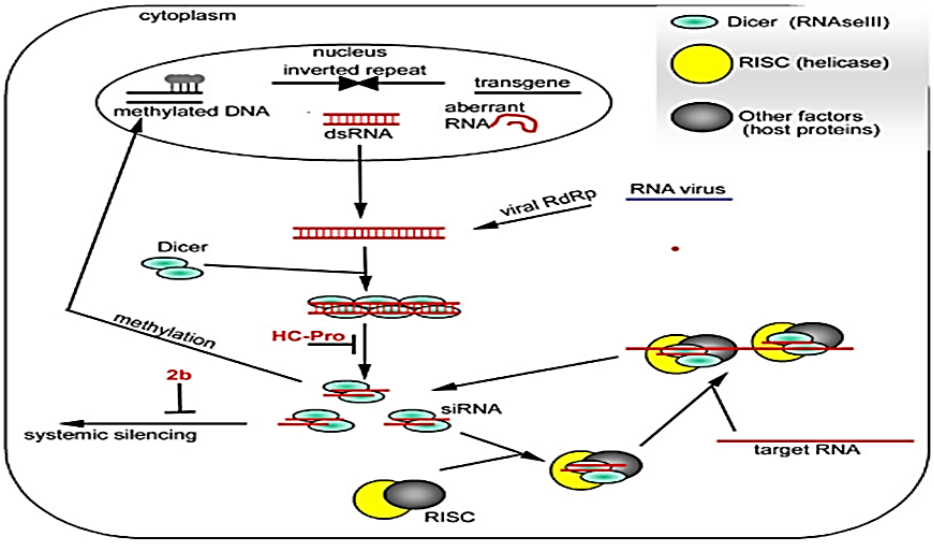

Figure 4.2: RNA silencing simplified model in plants [3].

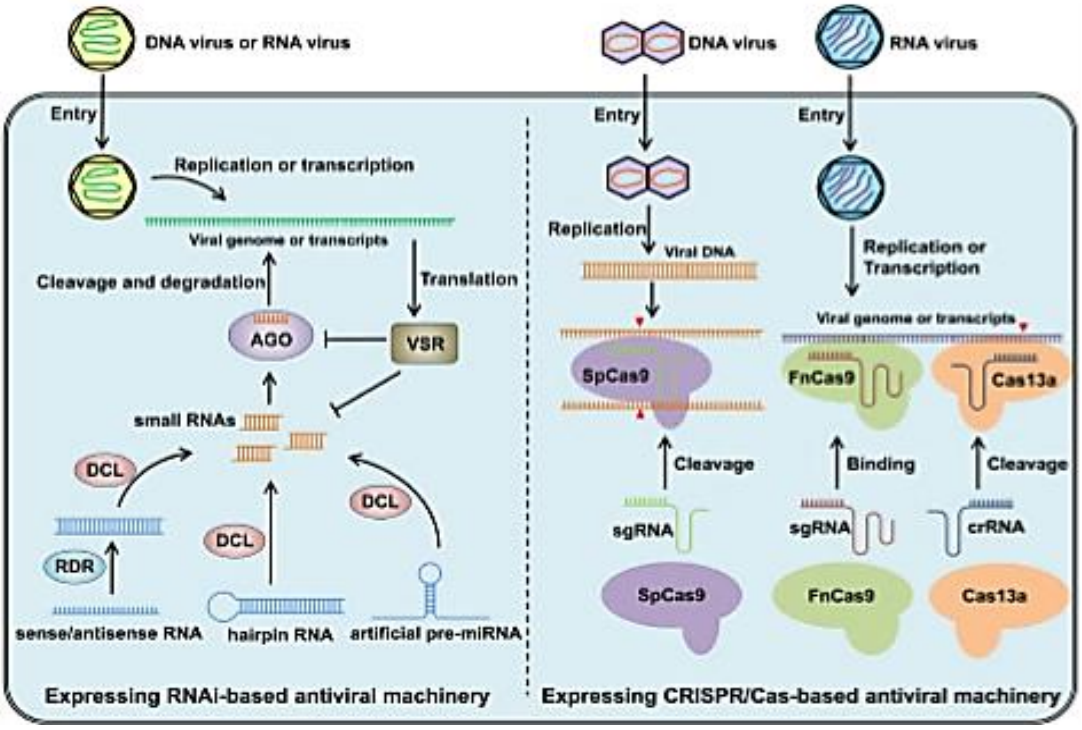

Figure 4.3: Schematic diagram describing the targeting of plant viruses by RNA silencing [6].

4.4 Identification of Microrna (amiRNA)

There are currently four approaches to the identification of miRNAs which are (1) genetic screening, (2) direct cloning after small RNAs isolation, (3) computational strategy and (4) sequence tags (ESTs) analysis.

I Genetic Screening Method

Initially, miRNAs were discovered through genetic screening. This method has been useful in detecting certain miRNAs, namely lin-4 and lin-7, but it has been limited because it is expensive, time consuming, and chance controlled.

II Direct Cloning After Small RNAs Isolation Method

To address some of the limitations of genetic screening, a new experimental method for isolating and identifying new miRNAs has recently been identified. This procedure necessitates the direct cloning of small RNAs after isolation. In this method, small RNA molecules are first isolated by size fractionation. The small RNAs are then ligated to RNA adapters at their 5′ and 3′ ends. Finally, they are reverse-transcribed to cDNA, which is then compressed and sequenced.

In comparison to general genetic screening, this method isolates and screens only small RNAs, making it a more efficient way to obtain miRNAs. The method has been used to discover new miRNAs in a variety of organisms, including animals and plants. This method has recently been refined by combining it with massively parallel signature sequencing (MPSS) for studying Arabidopsis miRNAs. This method is not only suitable for identifying miRNAs in plants, but it can also quantify miRNA abundance. Using this method, 77 of the 92 Arabidopsis miRNAs that were available in the miRNA database at the time of study were identified. This technique will allow for the analysis of additional miRNA discoveries until it is applied to other plant species.

III Computational Strategy Method

The third method is a traditional computational solution that focuses on a set of genomes. Several labs have successfully predicted miRNA genes in Arabidopsis, human, rice, C. elegans, and other animals using computational programmes such as MIRscan and MiRAlign. The actual computing approach was somewhat inefficient and certainly not comprehensive. Experiments such as cloning and Northern blotting will confirm the expected miRNAs.

IV Analysis of Sequence tags (ESTs)

The final method is an introduction to series tag (EST) expression analysis. Many miRNAs are known to change from species to species. It suggests an important method for predicting homologies of already established miRNA ororthologists. Weber (2005) used this theory to discover 35 existing human genes from mice, as well as 45 additional, putative miRNA genes. More specifically, this method is very useful in predicting miRNAs in multiple organisms, particularly those with unknown genomes. This has recently established an analytical approach to the EST to classify miRNA homologies. Since mining a freely available pool of ESTs, it is possible to classify 481 miRNA homologues previously identified in one plant species (Arabidopsis) but not in others. This suggests that EST analysis is a viable alternative method for identifying miRNAs, particularly for species with poorly understood genomes. This method, however, can identify only conserved miRNAs. Because most miRNAs are likely unconserved, the EST approach cannot be used to find these genes.

4.5 Artificial Mirna-Induced PTGS (AMIR)

Biotechnology has been developed for several perennial plants that target sRNA for pathogenic crop resistance, and then some virus-resistant crops have been controlled for commercial purposes. Artificial PTGS (AMIR-PTGS) induced by miRNA has resulted in antiviral silencing in perennials. Artificial miRNAs (amiRNAs) have also been developed to generate virus resistant plants and may further lead to the silencing of highly specific target genes. The amiRNA strategy for achieving transgenic virus-resistant plants has worked against a variety of viruses, including tomato leaf curl virus New Delhi virus (ToLCNDV), cucumber mosaic virus (CMV), potato virus X (PVX), potato virus Y (PVY), cotton leaf curl Burewala virus (CLCBV), and watermelon silver mottle virus (WSMoV).

4.6 Application Of miRNAs In Plant

Modern plant-associated biotechnologies, such as RNA interference (RNAi) antisense suppression, virus-induced gene silencing (VIGS), and transcriptional gene silencing (TGS), are now used in antiviral plant biotechnology. Furthermore, artificial miRNA (amiRNA) is another stable used in biotechnology throughout gene silencing plants, and amiRNA engineering has been widely used in various plants for targeted endogenous gene

regulation. MiRNA, a host-derived endogenous counterpart, has been widely used as a structural backbone to replace the current miRNA sequence with an area complementing the viral genome target due to its stability and efficiency.. Furthermore, multi-target miRNAs may have an impact on multiple viruses at the same time. MiRNA enzymes containing and engineered complementary sequences with Turnip yellow mosaic virus (TYMV) and transgenic Arabidopsis expressing recombinant miRNA precursors, for example, demonstrated actual virus resistance. Sun et al. (2016) recently developed three dimeric amiRNA precursor expression vectors based on the precursor structure of osa-MIR528 to approach the 3-proximal portion of RSV and Rice black streaked dwarf virus (RBSDV) CP genes. At low temperatures, the transgenic rice plants demonstrate high resistance to RBSDV and RSV infection. Thus, amiRNA engineering for antiviral resistance has been successfully used in a variety of plants, including benthamian, rice, wheat, maize, tomato, grapevine, and N. Arabidopsis. AmiRNA biology, along with bacteria and fungi, was widely used in plant tolerance to certain pathogens, in addition to being used in antivirals of plant immune systems. These findings suggest that amiRNA plant biotechnology could be extremely beneficial in increasing plant resistance to pathogens.

4.7 Advantage Of amiRNA (amiRNA) Virus Resistant

The development of artificial microRNAs (amiRNAs) provides a more detailed targeted strategy and has several advantages over other methods for RNA silencing. The amiRNAs are based on an endogenous miRNA precursor structure, and in relation to the target sequence, the endogenous miRNA region is replaced by the desired miRNA sequence. With the high specificity of the miRNAs transacting plant, undesirable off-target effects can be further avoided, allowing their silencing movement to be transmitted to future generations indefinitely. Furthermore, the small size of amiRNA allows multiple and unrelated amiRNAs to be included in a single cassette, which can then be used to create transgenic plants resistant to one or more simulated viruses. It was reported that the impact of amiRNA resistance would be more effective than that obtained from simple terms hairpin RNA24. amiRNAs are an alternative gene silencing method that has all of the advantages of previous PTGS-mediated silencing methods as well as additional specificity and durability advantages. Experiments with amiRNA-mediated gene silencing in mosses (Physcomitrella patens), monocotyledone (rice), algae (Chlamydomonas reinhardtii), and dicotyledonous plants were all successful (Arabidopsis, tomato, tobacco). The preventive feature of amiRNA in defence against viruses is an advantage, which means that the plant can be resistant to viruses without ever encountering them. AmiRNAs can be configured and optimised to silence one or more genes with related sequences, including tandem genes, with the same specificity as natural plant miRNAs, but without secondary siRNAs or non-autonomous effects. In the last decade, homology-dependent RNA-mediated silencing was commonly used for plant antiviral resistance via the expression of pathogenic sequences in double-stranded forms. RNA silencing, on the other hand, would suppress both invading viral RNA and virus-derived transgenic RNA, resulting in high resistance or deferred (recovery) resistance to virus problems. When compared to long RNA-mediated antiviral silencing, amiRNA has several advantages in terms of viral protection. To begin, there is no need for a long viral cDNA fragment to avoid off-target effects, and a short amiRNA sequence can be chosen without extensive homology to any plant genes. Second, the amiRNA-mediated approach has no environmental biosafety issues when used in agriculture. Focus on developing plants with viral sequences that may recombine with non-target viruses, but do not apply to plants expressing amiRNAs.

4.8 Advantages of Virus-Resistant in Plants

Plant diseases, as disruptive pathogens, cause significant crop damage by reducing crop quality, yield, and vigour. The development of virus-resistant crops appears to be a highly effective strategy for eradicating viral damage. There are potential environmental safety concerns regarding the constitutive in strategies where virus-derived genes are expressed in transgenic plants. The infectious virus should be capable of interacting with the appearance product of an inoculated milder virus strain in transgenic plants, thereby altering the existing biological properties of the virus and, ultimately, creating new virus species with new pathogenic properties, host range, and transmission specificities. From a different perspective, reasonable practicable results of recombination and hetero-encapsidation are also reported in conventional plants with combined viral infection. As a result, they are not limited to transgenic animals. And therefore, they are not concentrating on transgenic plants that are resistant

to viral infection. Thus, when assessing the environmental risks of virus-resistant transgenic plants, the primary focus should be on the consequences of recombination and hetero-encapsidation rather than their occurrence. The benefits of virus resistance include reduced time, cost, efficiency, breadth, and durability..

I Time Required

One of the most significant advantages of genetic transformation is its speed, and methods for resistance transgene design are becoming more common, at least for RNA viruses, though there is always room for improvement. In practise, however, the overall duration of the VRTP development process may not be that much slower, as it is possible for VRTPs to add a number of years to backcross the transgene from the initial transformant into the interesting elite lines, as well as to pass through the regulatory process for commercial release authorization.

II Cost

In cases where the virus's annual impact on a specific crop and continent is on the order of billions of dollars, such as the cassava mosaic disease reported in Africa, relatively expensive resistance methods may be considered for implementation. Given the magnitude of this particular disease problem, the only reasonable conclusion would be to continue advancing all potentially viable strategies involving both traditional transgenic and breeding resistance. VRTPs can be produced with remarkably simple technical expenditure in the simplest cases, and thus the hidden expenses are far from negligible at minimal cost. Many crops' elite breeding paths are not easily transformable, so it may take a lengthy series of backcrosses to actually bring the transgene to the most desirable plant genotypes. Furthermore, the time and effort required for the regulatory process is an additional expense, and one that almost everyone considers to be extremely discouraging in the development of any type of GM crop.

III Efficacy and Breadth of Resistance

The scope of resistance provided by various sources of natural immunity or resistance to viruses can vary greatly; in some cases, this can be as broad as strain-specific gene-for-gene resistance. Inoculating Virus-Resistant Transgenic Plants (VRTP) with a variety of greenhouse strains provides some indication of resistance breadth, but this is not always predictive of what will happen in the field. In the greenhouse, for example, a promising African cassava mosaic virus (ACMV) resistance proved ineffective. Long-term experience is also in short supply. Moreover, several related virus species are responsible for some viral diseases. This is true of cassava mosaic disease. Grapevine fanleaf disease and cotton leaf curl disease Obviously, such a case poses significant challenges to the development of VRTP in terms of resistance width and effectiveness.

IV Durability

Durability in any resistance that has not been fully characterised can be strongly related to issues of effectiveness and resistance width; in particular, if a resistance is strain-specific, doubts about its durability can be expressed.

4.9 Effects on Virus Resistances on Today Society

There are numerous developments that will have an impact on how we develop solutions and deploy traits. Combining multiple traits in one place is important, especially at a specific locus within the genome. This method poses a precise technological challenge to be developed that incorporates multiple features on a single disorder.

4.9.1 Development Factors

A First Development

As a result, the technological progress made for the production of cassettes containing multiple traits is a significant development. This is already possible to some extent, as evidenced by the identification of P. infestans with gene stacks containing three NLRs (Fig. 4.4) and five R-genes stack wheat toward stem rust. Recent technological advances, such as Nucleic Acid Transfer Gene Assembly in Agrobacterium Using Recombinase Technology (GAANTRY), have made it possible to produce capsules with up to ten traits in a total capacity of 28.5 kbp, whereas producing capsules with few times large inserts has historically been difficult. So,it is now technically feasible to create a capsule that can efficiently target one or two key viruses. Following that, because traits are prominent, mating can be used to create combinations. A marketable row of maize classified for its exchange term Smart Stax TM is an example of expression through that type of strategy. Four different biotech maize lines were crossed to create this line, which resulted in the synthesis of two insecticide resistance traits and six Bt traits, as well as lepidopteran insects and pollination. However, it will be a critical growth in the ability to produce massive piles of combined genes

in the coming years. For gene stacks to be useful, the triggering genes underlying the resistance must be identified. For many plants, cloned-resistance gene pools are still limited [4].

Figure 4.4: Introduced as a single breeding form in Uganda and Kenya, 3R potato comprises three NLRs that are efficient against Phytophthora infestans.

B Second Development

The second advancement is the use of low-cost sequencing techniques in conjunction with bioinformatic methods to classify genes with causal resistance even faster. This recognition can be performed in dynamic genotypes such as cereals and potatoes, as well as wild plant families such as pigeon peas [7]. Furthermore, the opportunity to obtain a high-quality assembly of the reference genome is now limited to standard practice. With ever-decreasing sequencing costs and increased processing capacity, these measures will soon become commonplace. This ability is significant because it allows scientists to study crop relatives in rich environments. Nature has had centuries to investigate and select resistance methods in order to provide a power of potentially verified strategies. Using an inexpensive, powerful sequencing power, wild germplasm will be processed for the dissemination of resistance traits at the point of origin. As many bacteria and viruses co-developed with wild progenitor species, an over-representation to a specific disease from resistance trait, which is especially successful at a low cost to the host which is indicated by the source of a wild parent.

C Third Development

A third advancement is the miniaturisation of sequencing technologies. A portable DNA sequencer has already been used to successfully identify pathogens and study the microbiome [8]. Real-time monitoring of pathogen populations in the field would be feasible and likely to be sufficiently structured for farmers or agronomists to do so within the time frame in which almost all of the solutions identified today enter the field. A thorough understanding of pathogenic population structure and dynamics can help determine the best response strategy, whether genetic or otherwise, against a given disease, such as identifying key effectors.

4.9.2 Environmental factor

The production of the virus, as well as its proliferation and spread within plants, have a direct effect due to environmental factors. The appropriate humidity and temperature for host growth and vector behaviour must be chosen [9]. Solanum melongena at a temperature range of 20–25 ° C is one of the examples that shows that lowering the temperature and humidity will increase the overall silencing capacity [10]. Viruses cause gene silencing (TRV-VIGS) Tobacco rattle virus has a significant silencing effect in tomatoes below 21 ° C and is inhibited at temperatures above 28 ° C; normal wheat growing temperatures at which the silence an endogenous (PDS) gene is silenced are between 18–22 ° C. Some satellite variants, such as DNA and DNA1, have a broader spectrum of temperature agility and can effectively induce gene silencing in the 22–32 ° C temperature range [5].

4.9.3 Virus Induced Gene

Recently, RNA silencing resistances have been widely used in papaya coat protein, plum pox virus, cucumber mosaic, and watermelon mosaic potyvirus 2 as well as major economic crops to work on specific genes. Stable and highly efficient technological (virus-induced gene) VIGS systems, as well as their implementation, are even more critical for long-lived fruit and forest trees. Meanwhile, one of the patterns of future research is the development of a VIGS system suitable for initial plant growth. Furthermore, the VIGS vector can induce genetically inherited transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS) to poorly control the gene expression liable for advantageous characteristics, or those converting information into key enzymes code involved in biological processes to rapidly upgrade significant economic characteristics of crops. VIGS also demonstrates its ability to be used effectively in the field of molecular genetic engineering. The research and development of more efficient, balanced, and ideal VIGS markers, as well as non-toxic and practical screening indicators for various plants, will continue [5]. Because VIGS technology does not require plant conversion and can thus be easily applied to stems, leaves, flowers, fruits, and other organs, research into fruit production and any traits related to fruit, or its kind, is extremely beneficial. In the future, VIGS

technology will be used more broadly in valuable crop production, including plant growth, maturation, quality, and practical yield gene studies [9]. VIGS may also work on bacterial, fungal, nematodal, and viral pathogenic genes to develop new plant defence strategies (Song et al., 2014). Furthermore, in order to create VIGS cDNA databases for high-level screening and usable analysis of plant genes, VIGS technology must be integrated in real time with molecular genetics techniques [11].

4.10 Future Challenge of Virus Resistance

Modern biotechnology implementation has a significant ability to overcome the limitations of traditional virus resistance propagation. First, because both RNAi and genome editing technologies require only viral sequence information, these approaches are particularly applicable to crops with limited genome sequence information. Second, using RNAi or genome editing to breed resistance does not require genetic crosses or the collection of separating progenies. As a result, the breeding period may be significantly reduced. Exogenous application of dsRNA for certain viral pandemics may provide a rapid emergency response to induce RNA silencing against the virus. Even so, there are some disadvantages to these modern innovations. Plant viruses have evolved a number of defensive measures against RNA silencing over time, one of which is the encoded viral suppressors of RNA silencing (VSR). Crop plants are also infected with a variety of viruses. Untargeted virus VSRs can disrupt the RNAi-mediated silencing process by targeting key components of RNAi pathways, and the targeted virus can occasionally have a strong VSR that can overcome RNA silencing immunity. The maximum ability of RNAi for engineering resistance to eukaryotic viruses has yet to be exploited, which has some advantages and disadvantages. More research is needed to improve these virus interference systems, such as increasing their durability, convenience, and delivery safety. The combination of RNA silencing techniques has enormous potential in antiviral breeding to overcome the shortcomings of each technique.

.

CONCLUSION

Essentially, the first thing that can be concluded from this study is that there are a few methods that will be useful in combating viruses, bacteria, and fungi, and to be more specific, the performance of virus resistance is clearly explained in terms of RNA gene silencing. There are two kinds of RNA silencing: siRNA and miRNA. Both resistances differ slightly but share a common characterization and function. This virus resistance is critical today because many farmers are struggling to maintain and sustain their agricultural development. A few advantages to remaining sustainable are the short time required, the need for simple technical expenditure, the effectiveness level reaching the sustainable demand, and the unquestionable durability. With all of the previously mentioned applications, benefits, and effects of RNA gene silencing, it will provide a comprehensive picture of how the gene can modify and manage viruses that attack crops. As a result, this virus resistance, which is RNA gene silencing, will have a significant impact on agricultural growth in accordance with sustainable strategies. A follow-up study is required from time to time.

Reference

[1] Cheeseman, J. (2016) 7 - Food security in the face of salinity, drought, climate change, and population growth. In Halophytes for Food Security in Dry Lands, 111–123.

[2] Legg, J.P., Shirima, R., Tajebe, L.S., Guastella, D., Boniface, S., Jeremiah, S., Nsami, E. et al. (2014) Biology and management of Bemisia whitefly vectors of cassava virus pandemics in Africa. Pest Manag. Sci. 70, 1446–1453

[3] Yaling Zhao, Xin Yang, Guohui Zhou, Tong Zhang. (2019). Engineering plant virus resistance form RNA silencing to genome editing strategies. Plant Biotechnology Journal, 1-9.

[4] Archana Singh, Irina Mohorianu, Darrell Green, Tamas Dalmay, Indranil Dasgupta, Sunil Kumar Mukherjeed (2019). Artificially induced phased siRNAs promote virus resistance in transgenic plants. Virology Article. 537:208-215

[5] Song Z, Li Z, Zhou C. (2014). Research advances of virus-induced gene silencing (VIGS). Acta Hortic ;41:1885–94.

[6] Devi Singh, Sarika Chaudhary, Rajendra Kumar, Preeti Sirohi, Kamiya Mehla, Anil Sirohi, Shashi Kumar, Pooran Chand and Pankaj Kumar Singh. (2015). RNA Interference Technology — Applications and Limitations. DOI: 10.5772/61760

[7] Kawashima CG, Guimaraes GA, Nogueira SR, MacLean D, Cook DR, Steuernagel B, Baek J, Bouyioukos C, Do Vale Araujo Melo B, Tristao G etal. (2016). A pigeon pea gene confers resistance to Asian soybean rust in soybean. Nature Biotechnology 34: 661–665.

[8] Hu Y, Green G, Milgate A, Stone E, Rathjen J, Schwessinger B. (2019). Pathogen detection and microbiome analysis of infected wheat using a portable DNA sequence. BioRxiv. doi: 10.1101/429878.

[9] Zhang X, Ji N, Min D, Shao S, Li F. (2017) Progress of research on application of VIGS vectors in fruit trees. J Fru Sci; 34:507–14

[10] Ji N-N, Min D-D, Shao S-J, Li F-J, Zhang XH (2016). Progress of research on application of VIGS vectors in vegetables. Plant Physiol; 52:810–6.

[11] Baohong Zhang, Xiaoping Pan, George P.Cobb, Todd A.Anderson. Plant microRNA: A small regulatory molecule with big impact. Development Biology.Volume 289: Pg 3-16