

SUGARCANE DERIVED FROM RNA INTERFERENCE AND PATHOGEN DERIVED RESISTANCE APPROACHES TO SUGARCANE MOSAIC VIRUS COMBINATIONS

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ABSTRACT

Sugarcane streak mosaic virus (SCSMV; Poacevirus; Potyviridae) causes streak mosaic disease in sugarcane (Saccharum interspecific hybrids), a major industrial crop widely cultivated for sugar and ethanol production in tropical and subtropical regions. Sugarcane mosaic virus (SCMV) is a serious disease that affects monocotyledonous plants, including sugarcane, and causes growth and productivity to decline. RNA interference (RNAi) is a method for controlling viral infection in plants that inhibits gene expression through RNA-mediated sequence-specific interactions. Sugarcane is related to Sorghum bicolor and Zea mays and belongs to the Poaceae family, genus Saccharum. Sugarcane is a monocotyledonous, tall, perennial, and economically important true grass that is cultivated in tropical and subtropical regions, producing two-thirds of the world's sugar, a 143-billion-dollar industry. Sugarcane mosaic virus (SCMV), Sugarcane streak mosaic virus (SCSMV), and Sorghum mosaic virus are among the viral diseases that affect it (SrMV). The aim of the research is to study use of an RNAi approach to control SCSMV in sugarcane. The current study on SCSMV disease control in sugarcane will provide a foundation and significantly broaden the scope of virus disease control using an RNAi approach.

KEYWORDS: Disease, Sugarcane, Control, RNA, Mosaic, etc.

1. INTRODUCTION

Plants frequently experience different microbes (microscopic organisms, growths, infection, and phytoplasma) attacks and show easily affected reaction through a progression of obstruction systems. Among them, infection disease is a significant danger to crops worldwide with loss of billions of US dollars in horticultural profitability consistently. To shield from viral diseases, plants have created

microbe explicit safeguard systems either through pathogenesis-related (PR) proteins or by RNA interference intervened quality quieting.

Infections are a significant danger to agribusiness everywhere on the world. Up till now, in excess of 1200 plant infections have been accounted for which incorporate 250 of those infections that cause significant misfortunes in crop yield. In nature, viral

particles exist as commit parasites which comprise of innate material pressed in a thick layered cover and totally rely upon have cell for a mind-blowing duration cycles. Infections uses have assets like nucleic corrosive, amino acids and certain proteins for their replication and endurance, hence upsetting host plant digestion to an impressive degree. The majority of the contaminating plant infections are as RNA infections like sugarcane mosaic infection, potato infection Y and so on in a tainted plant, infection amassing goes higher with expanded offspring rate through its replication. The spread of the infection in a plant is accomplished through its development from contaminated cell to solid one by means of plasmodesmata while significant distance development happens through phloem. Section of infection in plant ordinarily happens through physical injury like injury and so on or by means of certain viral vectors like aphid, flies and so forth.

RNA interference, often known as RNAi, is a biological process in which RNA inhibits the expression of genes. RNAi is usually triggered by foreign DNA and double-stranded RNA (dsRNA), which is found in parasitic and harmful (viral) RNA. RNAi is a biological system that is found in all eukaryotic cells, including plants. Several enzymes and proteins in a cell are involved in RNA processing and can control the RNAi pathway.

2. SUGARCANE MOSAIC VIRUS (SCMV)

Sugarcane mosaic infection (SCMV), having a place with class Potyvirus, family Potyviridae, is a genuine microbe of numerous monocotyledonous harvests including sugarcane. In Pakistan, roughly 10–32% misfortunes have been assessed in sugarcane yield which brings about 6–10% decrease in sugar creation. Contaminated plants indicated mosaic example and are described by average pinwheel formed incorporation bodies in cell cytoplasm. The 10 kb single abandoned RNA genome of SCMV encodes a solitary polypeptide which is severed either co-or post translationally into ten develops proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP).

Sugarcane mosaic virus (SCMV) is a devastating disease that affects monocotyledonous plants, particularly sugarcane, and causes growth and productivity to decline. RNA interference (RNAi) is a method for controlling viral infection in plants that limits gene expression through RNA-mediated sequence-specific interactions. The coat protein (CP)-encoding SCMVCP gene was introduced into the pGreen-0179 plasmid in both sense and antisense orientations in this work. To drive the transcription of the intron-hairpin constructs, HpSCMVCP-CaMV and HpSCMVCP-Ubi, respectively, Cauliflower mosaic virus (CaMV) and Zea mays ubiquitin (Ubi) promoters were chosen. Agrobacterium-mediated transformation was used to create transgenic sugarcane that expressed these constructs.



Figure 1: Sugarcane Mosaic Virus

SCMV (Sugarcane Mosaic Virus) is a plant pathogenic virus belonging to the Potyviridae family. The virus was initially discovered in Puerto Rico in 1916, and by the early 1920s, it had spread throughout the southern United States. SCMV is a major source of worry due to its significant economic impact on sugarcane and maize. Sugarcane mosaic virus infects sugarcane, maize, sorghum, and other poaceous plants, causing mosaic symptoms. This is the most common virus in sugarcane, with 21 types discovered in the United States.

2.1 Symptoms

Sugarcane mosaic virus symptoms include strong mottling throughout the plant's laminar zone, as well as yellowing of the leaves and growth retardation. Sugarcane mosaic virus is diagnosed by recognizing the infection's characteristic light green mosaic pattern, electron microscopy of leaf dips, and virus isolation and purification procedures. Mosaic is distinguished by its leaf symptoms. As with most sugarcane illnesses, the severity of the symptoms varies depending on the cane variety, growing conditions, and viral strain. Sugarcane mosaic, sorghum mosaic, maize dwarf mosaic, and Johnsongrass mosaic are the four different sugarcane "mosaic" diseases.

The mosaic virus causes the following symptoms:

- ✓ Yellow, white, or green stripes/ streaks/ spots on foliage
- ✓ Wrinkled, curled, or undersized leaves
- ✓ Prominent yellowing solely of veins
- ✓ Stunted growth and reduced yields
- ✓ Infected fruit is mottled and has elevated "warty" regions
- ✓ Dark green blisters
- ✓ Quickly drying stems

Plant viruses are difficult to identify since their symptoms are similar to those of other plants suffering from nutritional deficiencies, and they differ depending on the age of the plant at the time of infection. The virus can swiftly spread to an entire leaf, then the entire plant is infected, and the illness spreads to other plants in the area.

2.2 Spread of the Disease

SCMV is distributed in three ways: (1) through aphid vectors, (2) through contaminated seed

cane, and (3) through mechanical injection. SCMV can be transmitted from infected sugarcane to healthy sugarcane by a variety of aphid species. When vector populations are large, sensitive sugarcane cultivars are planted, and SCMV-infected plants are plentiful, mosaic spreads quickly.

2.3 Prevention and Control

Mosaic control is most successful when resistant cultivars are used. It is critical to plant mosaic-free seed cane. Grouping (digging up and destroying infected plants) is often not regarded a viable option when the infection level is high. Exceeds 5% of the population. Heat is used to control mosaic. Cuttings can be partially treated, but this is only practical in quarantine settings.

Although there is no cure for the mosaic virus, the following 10 precautions will help you avoid it:

- Fungicides will NOT cure this viral disease.
- Plant resistant kinds when they become available, or get transplants from a trustworthy supplier.
- Do not save seeds from infected vegetables.
- To lower the number of disease-carrying insects, spot treat using non-toxic, natural pest control products such as Safer Soap, Bon-Neem, and diatomaceous earth.
- Harvest-Guard® row cover should be erected until bloom to keep insect pests away from vulnerable crops/transplants.
- Remove all perennial weeds within 100 yards of your garden plot using the least hazardous herbicides.

- Human action, tools, and equipment can all propagate the virus. To limit the danger of contamination, wash your hands frequently and disinfect garden tools, stakes, ties, pots, greenhouse benches, and so on (one part bleach to four parts water).
- Avoid working in the garden when it is wet (viruses are easily spread when plants are wet).
- Avoid using tobacco in close proximity to sensitive plants. Cigarettes and other tobacco products may be infected with the virus and can spread it.
- Get rid of any diseased plants. Composting is not permitted.
- In your garden, plant virus-resistant kinds. Tomatoes resistant to cucumber mosaic virus have yet to be created, however tomatoes resistant to tobacco mosaic virus may have some resistance to cucumber mosaic virus as well.
- Mosaic viruses are mostly transmitted by insects, particularly aphids and leafhoppers. To prevent these insects from infecting your plants, try covering them with a floating row cover or aluminum foil mulches. Take a look at our other aphid-controlling tips.
- Keep weeds under control. Some varieties may act as disease hosts, and when aphids and other insects feed on these plants, the viruses are transferred to your garden plants.
- To minimize seed-borne mosaic viruses, soak sensitive plant seeds in a 10% bleach solution prior to sowing.

2.4 Characteristics of sugarcane

The plants grow to be 2–6 m (6–20 ft) tall, with strong, jointed, fibrous stalks rich in sucrose that collect in the stalk internodes. Sugarcanes are members of the Poaceae family of flowering plants, which also includes maize, wheat, rice, and sorghum, as well as a variety of forage crops.

2.5 Country Wise Production and Distribution of Sugarcane around the World

Sugarcane is farmed in a wide range of tropical and subtropical countries, but Latin America and Southern and Eastern Asia are the main producers. Sugarcane production worldwide in 2008 was 1,558 million metric tonnes.

- **Brazil**

Brazil is the world's biggest sugarcane producer, accounting for 33% of global output. More than half of Brazil's sugarcane is grown in Sao Paulo and its surrounding areas.

- **India**

Sugarcane is mostly farmed on India's Gangetic Plain. Though sugarcane is grown throughout India, Uttar Pradesh, Bihar, West Bengal, Maharashtra, Andhra Pradesh, Punjab, Tamil Nadu, Karnataka, and Odisha are the most important states.

- **China**

China is the world's third largest sugarcane producer, accounting for 6.83 percent of global sugarcane production. Sugarcane is grown in the provinces of Kwangtugn, Kwangsi, Fukien, Chekiang, Hunan, and Szechwan

- **Thailand**

Thailand produced over 644 lakh metric tonnes of sugarcane in 2008, accounting for 4.13 percent of global production.

- **Pakistan**

Pakistan is ranked fifth in the world for sugarcane production, producing 3.52 percent of the world's sugarcane in 2008. Lahore, Lyallpur, Multan, and Sialkot are the sugarcane-producing areas of Pakistan.

- **Mexico**

Mexico is the world's sixth-largest producer of sugar cane. It produced 506 lakh metric tonnes in 2008, accounting for 3.25 percent of global output. Sugarcane cultivation is primarily concentrated in Mexico's regions of Nuevo Leon, Tamaulipas, and Vera Cruz.

- **Columbia**

Columbia is the seventh-largest producer of sugarcane in the world. In 2008, it produced 400 lakh metric tonnes of sugarcane, accounting for 2.57 percent of global production. Aside from the countries listed above, Australia, the United States, the Philippines, Indonesia, Cuba, Argentina, Egypt, Kenya, Fiji, and others are among the world's top sugarcane producers.

3. RNA INTERFERENCE APPROACH IN SUGARCANE

To deal with numerous infections, plants have created a variety of defence systems (bacteria, fungi, virus, and phytoplasma). RNA interference (RNAi)-mediated protection against viral infection was discovered to be one of the most important innate immune responses. Viruses develop suppressors of the host RNAi pathway as a counter-attack technique against

the host defence. MicroRNAs (miRNAs) are a common family of non-coding single-stranded RNAs that are engaged in the RNAi pathway, which regulates gene expression post-transcriptionally. Sugarcane streak mosaic virus (SCSMV) is a Potyviridae virus with a single-stranded positive-sense RNA genome that causes sugarcane mosaic disease. We computationally hypothesised and empirically validated the miRNA encoded by the SCSMV genome in stem-loop RT-qPCR with detection rate of 99.9%, and identified their potential gene targets in sugarcane in this study. These sugarcane target genes greatly expand future research into the function of SCSMV-encoded miRNA during viral pathogenesis and could be used as a new technique for sugarcane mosaic disease management.

Sugarcane mosaic virus (SCMV) is a causal agent that causes sugarcane to grow and produce less. The most popular methods for developing resistance against plant viruses are pathogen-derived resistance (PDR) and RNA interference (RNAi).

3.1 Mechanism of RNAi/PTGS

Plants have evolved RNAi (RNA interference) as a natural defence against viruses and possible transposons. It's a biological mechanism in which short RNAs degrade target sequences at the mRNA level on the basis of similarity, preventing target RNAs from being translated. Two functionally distinct RNAs have been identified in plants: microRNA (miRNA) and small interfering RNA (siRNA). MiRNAs are tiny dsRNAs with a length of 21-26nt that are genome coded and found in every cell. They were made up of a double-stranded stem area and a single-stranded loop region. The miRNAs are produced from endogenous hpRNA precursors and are mostly engaged in development regulation. SiRNAs, on the other hand, are made up of long dsRNA and play a role in RNA interference defence.

Plants have an immune system that targets viruses called RNAi. Long dsRNAs are created during viral infection from viral RNA replication intermediates, which serve as a substrate for the cytosolic endonuclease Dicer. These dsRNAs are recognized by Dicer and cleaved into duplex siRNA (21-25nt). The siRNA duplex is made up of two strands: a guide strand that is complementary to the target mRNA and a passenger strand that is not.

3.2 Systemic spread of RNAi

When RNAi is triggered in one part of an organism, such as a plant, a mobile signal is generated that travels from cell to cell and systemically throughout the organism, allowing the RNAi response to be seen in distant plant tissues. Within the plant, this silencing signal goes through plasmodesmata (intercellular gaps) or phloem (phloem). It's thought that the existence of a mobile signal contributes to the systemic spread of silence.

3.3 Effective RNAi inducers

Both sense- and antisense transgenes have been used to silence genes in plant cells. ShRNA is an RNA molecule that contains a sense strand fragment, an antisense strand fragment, and a brief loop sequence between the fragments, producing a tight hairpin turn, and can decrease the expression of target genes through RNA interference. ShRNA cassettes can be utilized to create more efficient silencing by incorporating a specific plant promoter and terminator sequences to control the production of inversely repeated parts of the dsRNA. After the shRNA cassette is delivered to the plant cells, dsRNA molecules with a single-stranded loop and a double-stranded stem region are produced. Furthermore, Dicer starts the RNAi process by using the stem region as a substrate. RNA silencing mediated by the use of shRNA

cassettes enables stable and heritable gene silencing because it uses a specific promoter to ensure that the shRNA is consistently produced. Another rationale for shRNA cassettes' increased silencing efficacy is because dsRNA is transferred to a later step in the silencing pathway, where it works as a substrate for Dicer (a RNaseIII-like enzyme), bypassing the step where dsRNAs require plant expressed RdRPs for synthesis.

4. RNA INTERFERENCE (RNAi)- MEDIATED RESPONSE AND APPLICATIONS

RNAi is a strategy wherein a dsRNA is utilized to quietness unequivocal elements of a gene that is helpful to secure the host creature against viruses and new nucleic acids. This component is delineated by various names in various living beings, for example, controlling, post-transcriptional gene hushing, and RNA interference in parasites, plants, and creatures, individually. Greater part of the plant viruses has auxiliary structure components in their RNA genome and produce dsRNA intermediates by viral RdRPs during replication. At that point, virus-determined little RNAs are created by RNA hushing framework with the assistance of dsRNA intermediates (VsRNAs). VsRNAs joining in RISC (RNA induced silencing complex) prompts the arrangement explicit degeneration of viral genome and inception of versatile quieting signal, which multiplies by means of plasmodesmata among cells and over exceptionally huge separations through a transfer intensification process partner have RdRPs.

4.1 RNA interference and respiratory viruses

Vaccines against specific viruses – especially respiratory coronaviruses – are often ineffective due to the high number of mutations that occur

to such viruses. Antiviral treatments often present with issues such as antiviral drug resistance.

Respiratory syncytial virus (RSV) is a negative-stranded RNA virus (a Paramyxoviridae) that causes bronchiolitis and pneumonia in children or older immunocompromised adults. RSV results in repeated re-infections throughout life, and to date, there is no vaccine for RSV. Cell studies have successfully used RNAi against RSV (targeted against its viral fusion and phosphoprotein) resulting in efficient inhibition and prevention of infection.

4.2 RNAi- Controlling Insect Vectors Transmitting Plant Viruses

The system of RNAi has been inspected in roughly thirty bug species from various requests of class Insecta. Two methodologies utilized for quieting creepy crawly vectors are hushing that prompted block with the transmission and the other one is concealment of the objective genes that prompts passing and consequently declining the bug populace.

For transmission and survival, the majority of plant viruses that cause illness in agricultural crops rely on biotic vectors. Insects are the most common vectors for plant viruses, although other vectors include mites, nematodes, and chytrid fungi. The review by Bragard and colleagues provides a complete picture of plant virus families and their known related vectors. Aphids, thrips, leafhoppers, planthoppers, and whiteflies are the most well-studied plant viral insect vectors. Non-persistent, semi-persistent, and persistent viral transmission by vectors occur when the transmission window to distribute the virus to a new host plant after the vector feeds on an infected plant lasts seconds to minutes, hours to days, or days to weeks, respectively.

5. VIRUS RESISTANCE IN TRANSGENIC PLANTS

At the point when a plant experiences virus, it responds normally through touchy reaction (HR) and outrageous resistance response (ER) which incites the creation of optional metabolites named as reaction components in plants. These reaction components incorporate raised degrees of ethylene, jasmonic corrosive, salicylic corrosive, nitric oxide and expanded pace of particle motion, in mix these variables block the virus passage and/or dispenses with the virus. The procured virus opposition components in plants are of two kinds: a) gene quieting autonomous virus obstruction and b) gene hushing trustworthy virus obstruction by means of Post Transcriptional Gene Silencing (PTGS).

The first incorporates coat protein-intervened, development protein-interceded and replicase protein-interceded obstruction, while second incorporates microorganism determined opposition, antisense RNA intervened obstruction and RNA-interceded obstruction. PTGS is a developmental moderated instrument in plants against possible damages by viruses and transposons. In this process, a plant safeguards itself by abusing the necessity of plant RNA viruses to imitate utilizing a twofold abandoned, replicative middle of the road (dsRNA).

Water, wind, insects, and humans can all help plant infections spread quickly and across long distances. Diseases are predicted to diminish plant yields by 10% every year in more industrialized nations or agricultural systems, but yield loss from diseases commonly reaches 20% in less developed settings. Disease control, on the other hand, is generally effective for most crops. Disease control is performed through the use of disease-resistant plants and plant cultivation techniques such as crop rotation, pathogen-free seed, optimum planting

date and plant density, field moisture control, and pesticide application.

The primary methodology made by plant agronomists was the vaccination of powerless plant with a milder strain of the objective virus. This strategy was named as cross insurance and was utilized on crops like tomato, papaya and citrus. Researchers were met with progress as significant opposition was accomplished in transgenic plants through work of this methodology however the achievement was gone with a significant disadvantage that the milder strain of the virus giving insurance to one yield may cause genuine diseases on assortments becoming close by.

- **Satellite RNA Mediated Resistance:**

It has been proven that some satellite RNA can reduce the symptoms of their helper virus. Satellite RNAs are RNA species linked to certain plant RNA viruses. Its interaction with the virus may or may not be necessary for replication. Satellites are replicated in cells that have been infected with a specific virus.

- **Ribozyme Mediated Resistance:**

Ribozymes are tiny RNA molecules generated from the satellite tobacco ringspot virus, also known as catalytic RNA (TRSV). These are also derived from viroids, including sat RNA viroids. Ribozymes catalyze the breakage of RNA in a specific manner. Ribozymes cleave particular target RNA (introns) intramolecularly in most cases.

- **Movement Protein (MP) Mediated Resistance:**

Only when plant virus is mobilized from the initial site of infection into nearby healthy cells can a successful viral infection be created. Plant viruses use a virally encoded protein called movement protein to travel from one cell to

another, according to recent research (MP). Plant vims (or nucleic acid) typically travel from cell to cell via plasmodesmata.

- **Replicase Mediated Protection:**

Non-structural mediated resistance is the name for this sort of defence. TMV replicase enzyme is likely responsible for the production of genomic and non-genomic RNAs in the genomic organization. The genomic RNA encodes 126 and 183 kDa proteins, which are thought to be components of the replicase and play a role in TMV genome replication. TMV has a third sub genomic RNA with nucleotide residue 3405 carrying open reading for a 54 kDa protein at its 5 terminuses. Plants transformed with the TMV non-structural gene sequence encoding the 54 kDa protein show significant resistance to TMV infection.

- **RNA Mediated Resistance**

RNA-mediated resistance is a unique approach that has been observed/adopted in plants and gives great viral protection. A study of RNA-mediated virus resistance and co-suppression has given researchers a better understanding of how plant viruses interact with their hosts.

6. SUGARCANE GENERATED FROM RNA INTERFERENCE AND PATHOGEN-DERIVED RESISTANCE APPROACHES TO COMBATING SUGARCANE MOSAIC VIRUS

SCMV (Sugarcane Mosaic Virus) is a virus that causes sugarcane to grow slowly and generate less sugar. Pathogen-derived resistance (PDR) and RNA interference are the most common ways for generating resistance against plant viruses (RNAi). Using PDR and RNAi methods, two varieties of transgenic sugarcane were created using a gene encoding SCMV coat protein (CP) (SCMVCp). The purpose of this study was to test how resistant the two

transgenic sugarcanes were to SCMV after being inoculated with the virus artificially. Transgenic sugar cane lines were used in the experiment, which were validated using PCR analysis. The insertion of the gene producing CP in the transgenic strains was confirmed by amplification of a 702 bp DNA fragment of SCMVCp. Mosaic symptoms appeared earlier in PDR transgenic lines, at 21 days after viral inoculation (dpi), but later in RNAi transgenic lines, at 26 days after viral inoculation (dpi) (dpi). Symptom analysis found that mosaic symptoms were present in 77.8% and 50% of infected plants in the PDR and RNAi transgenic lines, respectively. According to RTPCR investigations, the SCMV nuclear inclusion protein b (Nib) gene was amplified in the symptomatic leaves of plants classified as susceptible lines.

SCMV is a positive sense single stranded RNA (+ssRNA) genome type that belongs to the Potyvirus genus, which is part of the Potyviridae family. Among the 10 proteins encoded by the genome's open reading frame are P1, HCpro, P3, 6K1, CI, 6K2, NIaVPg, NIapro, NIB, and CP (ORF). During the feeding process, potyvirus is transferred to plants via stylets by vectors such as aphids. The virus can be spread in two ways. The coat protein (CP) interacts directly with binding sites (receptors) in the aphid stylet in the capsid method. By establishing a reversible molecular bridge between CP and aphid receptors, the non-structural protein HC Pro (Helper component proteinase) improves binding in the helper method. As a result, the CP is the most well-studied molecular infection in plants caused by the virus.

CP assists in the systemic spread of viruses in plant tissues by regulating the assembly process of intact viral particles. CP was known to confer resistance to Tobacco mosaic virus (TMV) infection in transgenic tobacco using the CP mediated resistance (CPMR) technique. This

method produces resistance when the Cp gene is expressed in plant cells and creates a CP aggregate. TMV virus infection has been shown to be suppressed by transgenic tobacco expressing the CP, which prevents virus particle assembly by interfering with the accumulation of mobility protein (MP), which is necessary for moving viral particles from cell to cell. If TMV virions display CP, they may be stopped from going through cotranslational disassembly, which is an early stage of infection. To avoid infection, transgenic CP uses a defensive mechanism that disassembles virus particles as quickly as feasible. Using CP, peanuts, eggplant, and sugarcane have all evolved virus resistance (*Saccharum officinarum* L.). Pathogen-derived resistance is a type of virus resistance that involves the creation of viral protein from nucleotide sequences received from viruses in plant cells (PDR).

RNA interference (RNAi), also known as RNA silence, is a defence mechanism that protects plants from pathogen infections by specifically downregulating viral gene expression. Transgenic RNAi has been created as a molecular technique for enhancing disease resistance in plants. The resistance mechanism of RNAi has efficiently created small interfering RNA (siRNA) to down regulate damaging fungal genes in *Aspergillus* and *Fusarium* fungus, based on the formation of hairpin RNA (hpRNA) to control mycotoxigenic fungi. Dicer enzymes, which are RNase III-like enzymes, convert dsRNA or hpRNA into small interfering RNA (siRNAs) of 21 to 28 nucleotides in length. An RNA-induced silencing complex is formed when siRNAs bind to AGO, a ribonuclease H-like protein (RISC). Small RNA complexes recognize RNA targets by complementary base pairs, whereas the AGO protein acts as an effector to change target activity. As a consequence, the RNAi resistance mechanism, which targets the Cp gene in Plum pox virus,

has a high efficacy in inhibiting viral infection in transgenic plants via dsRNA expression. In transgenic sugarcane, the RNAi mechanism has recently been found to be effective in preventing virus infection.

Transgenic sugarcane resistance to SCMV was achieved using PDR and RNAi methods targeting the coat protein (Cp) gene of SCMV. We used a viral challenge to test how effective the two types of transgenic sugarcane lines were at avoiding SCMV infection. In compared to the PDR method, the data showed that RNAi was more effective at preventing virus infection. This is the first study to compare the response to SCMV infection of two separate transgenic sugarcane lines generated utilizing the PDR and RNAi methods.

7. CONCLUSION

Sugarcane streak mosaic virus (SCSMV), a Poacevirus, is the causal virus causing mosaic disease in sugarcane in a number of Asian countries, with significant genetic heterogeneity. Despite the fact that the virus infects the crop with Sugarcane mosaic virus (SCMV), a Potyvirus, it outperforms SCMV in terms of propagation and titre. We've done extensive research to determine the functional activity of viral suppressors in the SCSMV genome. Resistance to SCSMV in sugarcane plants was obtained in this study by treating setts with bacterially-expressed dsRNA that was complementary to SCSMV's CP region. Total RNA was extracted from healthy and SCSMV-symptomatic sugarcane leaf samples to create a cDNA library. PCR amplification of the SCSMV coat protein domain revealed the presence of SCSMV. An expression vector (pFGC1008) was created by cloning the CP region of SCSMV in both sense and antisense orientation, bordering both sides of the GUS intron region, for the synthesis of SCSMV encoded dsRNA. Finally, the RNAi technique mediated by SCSMV encoded CP specific

dsRNA, as well as the sett treatment strategy used in this study, provide a useful tool for managing mosaic virus illness in sugarcane.

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