

RESEARCH ARTICLE

In silico identification of sugarcane (*Saccharum officinarum* L.) genome encoded microRNAs targeting sugarcane bacilliform virus

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Abstract

Sugarcane bacilliform virus (SCBV) is considered one of the most economically damaging pathogens for sugarcane production worldwide. Three open reading frames (ORFs) are characterized in the circular, ds-DNA genome of the SCBV; these encode for a hypothetical protein (ORF1), a DNA binding protein (ORF2), and a polyprotein (ORF3). A comprehensive evaluation of sugarcane (*Saccharum officinarum* L.) miRNAs for the silencing of the SCBV genome using *in silico* algorithms were carried out in the present study using mature sugarcane miRNAs. miRNAs of sugarcane are retrieved from the miRBase database and assessed in terms of hybridization with the SCBV genome. A total of 14 potential candidate miRNAs from sugarcane were screened out by all used algorithms used for the silencing of SCBV. The consensus of three algorithms predicted the hybridization site of sof-miR159e at common locus 5534. miRNA–mRNA interactions were estimated by computing the free-energy of the miRNA–mRNA duplex using the RNAfold algorithm. A regulatory network of predicted candidate miRNAs of sugarcane with SCBV—ORFs, generated using Circos—is used to identify novel targets. The predicted data provide useful information for the development of SCBV-resistant sugarcane plants.

1. Introduction

Sugarcane bacilliform viruses (SCBVs) are classified into the *Badnavirus* genus of the *Caulimoviridae* family. These viruses are composed of monopartite, circular, non-enveloped bacilliforms that are (30 × 120–150 nm) in size, with a double-stranded DNA (ds-DNA)- genome of approximately 7.2–9.2 Kbp in size [1]. The genome of SCBV constitutes three major open reading frames (ORFs) that are located on the ‘plus DNA strand’ with a single discontinuity [2]. ORF1 encodes a small hypothetical protein. ORF2 encodes a virion-associated DNA-

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binding protein. ORF3 encodes the largest polyprotein, represented as P3 here, and is composed of multiple functional sub-units. The polyprotein (P3) is cleaved by a viral aspartic protease to give rise to multiple functional small proteins, thereby forming intracellular movement, capsids, aspartic proteases, reverse transcriptase (RT), and ribonuclease H (RNase H) [1–6]. The RT-RNaseH-coding region is considered to be the most common taxonomic marker for the identification of badnaviral genomic components. This coding region is a standard source to compare the sequence diversity of the badnaviral genomes [7].

The first report of SCBV infection was observed in the Cuban sugarcane cultivar B34104 in 1985 [8]. These viruses have been disseminated worldwide and have reduced crop production significantly because of the accessibility and exchange of biological materials globally. SCBV is a source of infection for several bioenergy crop sugarcane cultivars, varieties, and species. The broad host range of the SCBV includes diverse and economically important members of the *Poaceae* (sugarcane, and rice) and *Musaceae* (banana) families. Natural transmission of SCBV is disseminated by sap-feeding mealybug species via vegetative cutting [9]. SCBV disease symptoms include chlorosis and leaf freckling. Infected sugarcane plants have also been monitored and feature no symptoms. In recent years in China, SCBV-infection in sugarcane plants has resulted in a reduced sucrose content, juice, stalk weight, purity, and gravity [6].

RNA silencing is an evolutionary conserved homolog-dependent regulatory mechanism of gene expression in all eukaryotes and is triggered by small RNA molecules (sRNA). dsRNA is the ultimate trigger of the RNAi complex, which works as a replication intermediate created by viral RNA-dependent RNA polymerases (RDRs) [10]. The RNAi mechanism works with cleavage of the precursor dsRNA into short 21–24 nt siRNA or miRNA duplexes using an RNaseIII-like enzyme called Dicer (DCL) [11–13].

The RNAi-mediated response of plants against invading viruses is especially significant during the infection period [14]. The RNAi mechanism inhibits protein translation at the mRNA level via a highly sequence-specific strategy [15]. Sugarcane has inherited an active immunity, consisting of small non-coding microRNAs (miRNAs) to control viral diseases. miRNA-mediated gene silencing is considered to validate the activity of positive or negative immune-based regulation; it is also considered a key activator of immune defense in plants [16, 17]. RNA silencing in the form of miRNAs within the host plant is a source of natural immunity. Such a mechanism provides resistance to the host plant after infection via foreign genetic elements, including plant viruses [18–20].

Artificial microRNA (amiRNA)-mediated RNAi produces a single 21-nucleotide amiRNA (analogous to a single siRNA) that only recognizes a target sequence with less than five mismatches. This feature not only ensures a higher silencing specificity for amiRNAs than hairpin RNAs but also offers unique advantages [21, 22]. amiRNA-mediated silencing of invading viruses in plants was first reported by Niu [23]. This amiRNA-based silencing strategy has been applied to with many plants in order to combat plant viruses, such as cotton leaf curl Kokhran virus (CLCuKoV) [24], cucumber mosaic virus (CMV) [25], cymbidium mosaic virus (CymMV), and odontoglossum ringspot virus (ORSV) [26].

In this study, we performed a comprehensive bioinformatics analysis to identify sugarcane miRNAs predicted to target the SCBV genome. Computational methods can determine how miRNAs target a desirable mRNA. A large number of computational algorithms are publicly available for miRNA target prediction. It is highly advantageous to acquire several computational tools with different features. Researchers are challenged with an important choice regarding selecting suitable tools for prediction [27]. The current study implements miRNA prediction algorithms and identifies potential targets of sugarcane-derived miRNAs against SCBV as a precedent for creating resistance in sugarcane cultivars using RNAi technology. Potential sugarcane miRNAs are also screened for understanding sugarcane–*Badnavirus*

interactions. The novel computational approach here supports the idea of generating SCBV-resistant sugarcane plants through genetic engineering.

2. Materials and methods

2.1. Retrieval of sugarcane MicroRNAs

Mature sugarcane microRNAs (miRNAs) and stem-loop hairpin precursor sequences were retrieved from the miRNA biological sequence database miRBase (v22) (<http://mirbase.org/>). miRBase serves as primary public repository and standard online reference resource for all published miRNA sequences, along with providing textual annotations and gene nomenclature [28–30]. In this study, 16 *S.officinarum* (MI0001756-MI0001769) and 19 *Saccharum* spp. (MI0018180- MI18197) miRNA sequences were downloaded (S1 Table in [S1 File](#)).

2.2. SCBV genome retrieval and annotation

The full-length transcript of the SCBV-BRU genome was isolated from the *S. officinarum* cultivar and then published, and available via accession no. JN377537 [31]. The expected sizes and abundances of the ORFs along nucleotide distributions of the above mentioned NCBI retrieved SCBV-BRU genome were estimated using the pDRAW32 DNA analysis software (version 1.1.129) (AcaClone software). The SCBV-BRU genome annotation represents ORFs of varying lengths.

2.3. Target prediction in SCBV genome

Target prediction is considered a key feature towards the identification of credible miRNA–mRNA interaction hybridization. At present, many target prediction algorithms have been designed to predict and identify the best miRNA target candidate. Each tool uses specific criteria and methods for miRNA target prediction. We used four target prediction algorithms cited in the literature (miRanda, RNA22, RNAhybrid and psRNATarget) to find the most relevant sugarcane miRNAs for silencing of the SCBV genome ([Table 1](#)). These computational tools compute the complementarity-based attachment of miRNA-mRNA. This attachment is divided into seed and mid regions. The mismatch in the seed region is more damaging than that of a mismatch in the middle region of miRNA-mRNA attachment. This provides the basis for over-sensitivity for the computation. We can set higher penalty of a mismatch in seed region which will make the prediction more sensitive. We designed an effective computational approach to analyze miRNA targets at three different prediction levels namely the individual, union, and intersection levels. A detailed workflow pipeline is presented in ([Fig 1](#)) below.

Table 1. Comparison of distinctive parameters used in the common target prediction tools.

Tools	Algorithms	Seed pairing	Target site accessibility	Multiple sites	Translation Inhibition	Availability
miRanda	Local alignment	+	+	+	+	Web server and source code
RNA22	FASTA	–	+	+	–	Only web server
RNAhybrid	Intermolecular hybridization	+	+	+	+	Web server and source code
psRNATarget	Smith-Waterman	–	+	+	+	Only web server
Tapirhybrid	FASTA	+	+	+	–	Web server and source code
Targetfinder	FASTA	+	–	–	–	Only source code
Target-align	Smith-Waterman	–	–	+	–	Web server and source code
Targetscan	Custom made	+	–	+	+	Only source code

‘+’ Represents a feature was used, ‘-’ indicates that a feature was not used.

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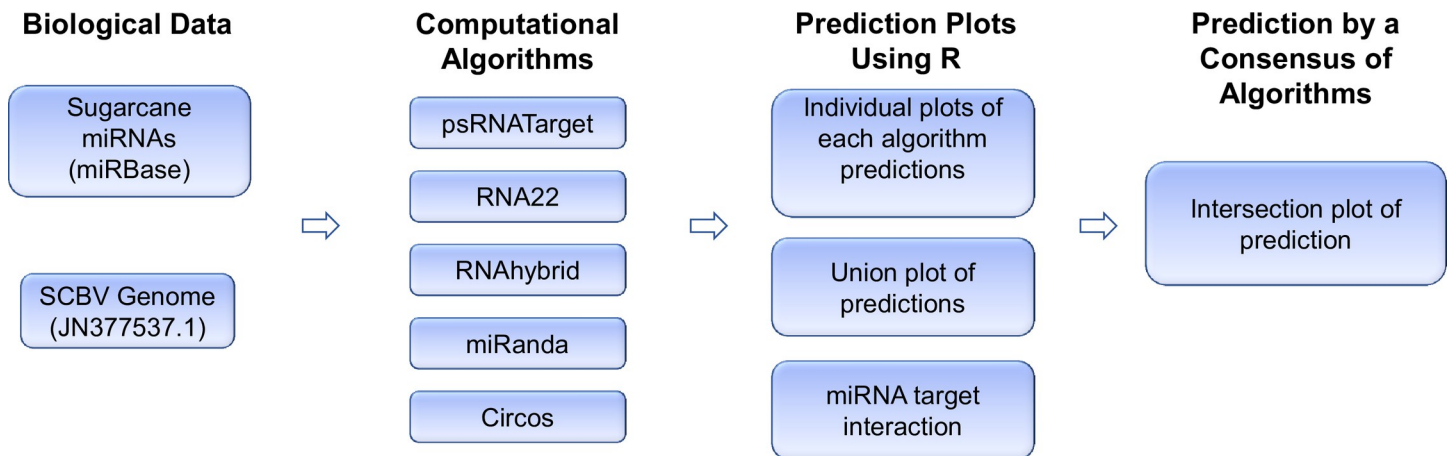


Fig 1. The methodology of host or sugarcane miRNA target prediction in the SCBV genome. A flowchart designed for predicting candidate miRNAs of host that could potentially target SCBV genome. The biological data are composed of sugarcane miRNAs retrieved from the miRBase database and SCBV genome from NCBI GenBank database. The algorithmic framework consists of three kinds of tools used for identification of sugarcane-encoded miRNA targets, prediction of secondary structures and visualization of mi RNA–target interaction. The R language was used to create plots and select data using in-house scripts/codes.

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2.4. miRanda

miRanda is considered to be a standard miRNA–target predictor scanning algorithm. It was implemented for the first time in 2003 [32] and has been updated into a web-based tool for miRNA analysis [33]. The latest version of the miRanda software was accessed using the online source website (<http://www.microrna.org/>).

2.5. RNA22

RNA22 is a user-friendly, web-based (<http://cm.jefferson.edu/rna22v1.0/>) novel pattern-recognition algorithm that is used for predicting target sites with corresponding hetero-duplexes. Non-seed-based interaction, pattern recognition, site complementarity, and folding energy are the key parameters of the RNA22 algorithm [34]. Final scoring removes the need to use a cross-species conservation sequence filter [35].

2.6. RNAhybrid

RNAhybrid is an easy-to-use, fast, flexible, web-based (<http://bibiserv.techfak.uni-bielefeld.de/rnahybrid>) intermolecular hybridization algorithm that is used to estimate mi RNA–mRNA interaction as well perform target prediction based on MFE hybridization. A p-value is assigned to assess RNA–RNA interaction-based hybridization sites in the 3' UTR sequence [36]. RNAhybrid is widely used to estimate the MFE of the consensual mi RNA–target pair and the mode of target inhibition as suggested [37].

2.7. psRNATarget

psRNATarget is a new web server (<http://plantgrn.noble.org/psRNATarget/>) that is used to identify the target genes of plant miRNAs based on a complementary matching scoring schema. It has been used to discover validated mi RNA–mRNA interactions [38]. The plant psRNATarget was designed to integrate a key function for miRNA target prediction using complementarity scoring and secondary structure prediction [39]. Target site accessibility was evaluated by estimating the unpaired energy (UPE) to unfold a secondary structure [37].

2.8. Mapping of mi RNA–target interaction

An interaction map was created between sugarcane miRNAs and SCBV ORFs using the Circos algorithm [40].

2.9. RNAfold

RNAfold is a new web-based algorithm and was applied for the prediction of the stable secondary structures of pre-miRNAs based on the MFEs [41].

2.10. Free energy (ΔG) estimation of duplex binding

RNAcofold is a novel web-based server (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAcofold.cgi>) that is used for estimating free energy (ΔG) associated with miRNA–mRNA interactions [42]. The free energy of miRNA–miRNA duplexes is considered a key predictor for miRNA targeting during hybridization.

2.10.1. *In Silico* sugarcane miRNA expression profiling. Plant miRNA Expression Atlas (PmiRExAt) is a web-based resource comprising a miRNA expression profile and searching tool for 1,859 wheat, 2,330 rice, and 283 maize miRNAs [43]. PmiRExAt can be accessed at <http://pmirexat.nabi.res.in/>. The sequences of mature microRNAs from sugarcane were blasted in PmiRExAt and the expression patterns of the homologous microRNAs were searched in wheat, rice, and maize.

2.10.2. Graphical representation. All the computational data were processed into graphical representations using R version 3.1.1 [44].

3. Results

3.1. Genome Organization of SCBV

SCBV is a plant pararetrovirus that is, classified in the genus *Badnavirus* of the family *Caulimoviridae*. The genomic ds-DNA molecule of SCBV is comprised of three ORFs, separated by an intergenic region (IR). ORF1 is composed of 557 nucleotides (618–1175 nt), encoding a hypothetical protein (P1) with 185 amino acids (aa), while ORF2 is composed of 370 nucleotides (1176–1546 nt) codes for a virion-associated DNA binding protein (P2) with 123 aa. The precise functional capabilities of these proteins (encoded by ORF1 and ORF2) have not been explored. A large polyprotein (1977 amino acids) is encoded by ORF3 (1547–7479 nt) to cleave by a viral aspartic protease. The resulting proteins obtained are named as movement, capsid protein, aspartyl proteinase, reverse transcriptase and ribonuclease H. The IR is composed of 1022 nucleotides (7479–618) and is located between 3'-ORF3 to 5'-ORF1. The intergenic region (IR) works as a promoter and controls the transcription and regulation of the SCBV genome. The genome organization of the SCBV with three ORFs is shown in (Fig 2).

3.2. ORF1-encoding hypothetical protein

The hypothetical protein of the SCBV genome that is encoded by ORF1 had an unknown function [2]. In miRanda that only predicted two target sites for sugarcane miRNAs sof-miR156 and sof-miR168 at nucleotide positions 818–837 and 617–638 to target ORF1 (Fig 3A). RNA22 predicted the binding sites of miRNAs sof-miR156 and sof-miR168a at the two different locus positions of 817 and 834, respectively (Fig 3B). The RNAhybrid algorithm predicted multiple potential binding sites of sugarcane miRNAs sof-miR168 (a, b), ssp-mi827, and ssp-miR1128 at nucleotide positions 612–632, 1170–1192, and 1137–1157 respectively (Fig 3C). In addition, psRNATarget identified potential hybridization sites of sof-miR159 (c, e) at locus positions 1003 and 820 respectively (Fig 3D).



Fig 2. Genomic organization of the sugarcane bacilliform virus. The predicted ORFs denoted with arrows are composed of dsDNA that is 7884 bp in size.

<https://doi.org/10.1371/journal.pone.0261807.g002>

3.3. ORF2 encoding DNA binding protein

A nucleic acid (DNA)-binding protein of the SCBV genome is encoded by ORF2 [6, 45]. RNAhybrid and miRanda predicted potential target binding site of ssp-miR166 at locus position 1449–1470 (Fig 3A and 3C). Suitable candidate miRNAs from sugarcane (ssp-miR444 (a, b, 3p) were observed to target ORF2 at a single loci nucleotide position (1301–1326) as determined by the miRanda algorithm (Fig 3A). No sugarcane miRNAs were predicted to target the ORF2 gene with the RNA22 tool (Fig 3B). Similarly, RNAhybrid predicted the binding of ssp-miR166 at locus 1450 (Fig 3C). The miRNA prediction results revealed that no candidate miRNA was identified to have a potential genome binding site in the ORF2 region, as predicted by psRNATarget (Fig 3D).

3.4. ORF3 encoding polyprotein (CP, AP, RT, and RNase H)

The poly proteins constitute the largest portion of the SCBV genome encoded by ORF3 [2, 6]. Potential candidate miRNAs from sugarcane were identified by the miRanda algorithm to

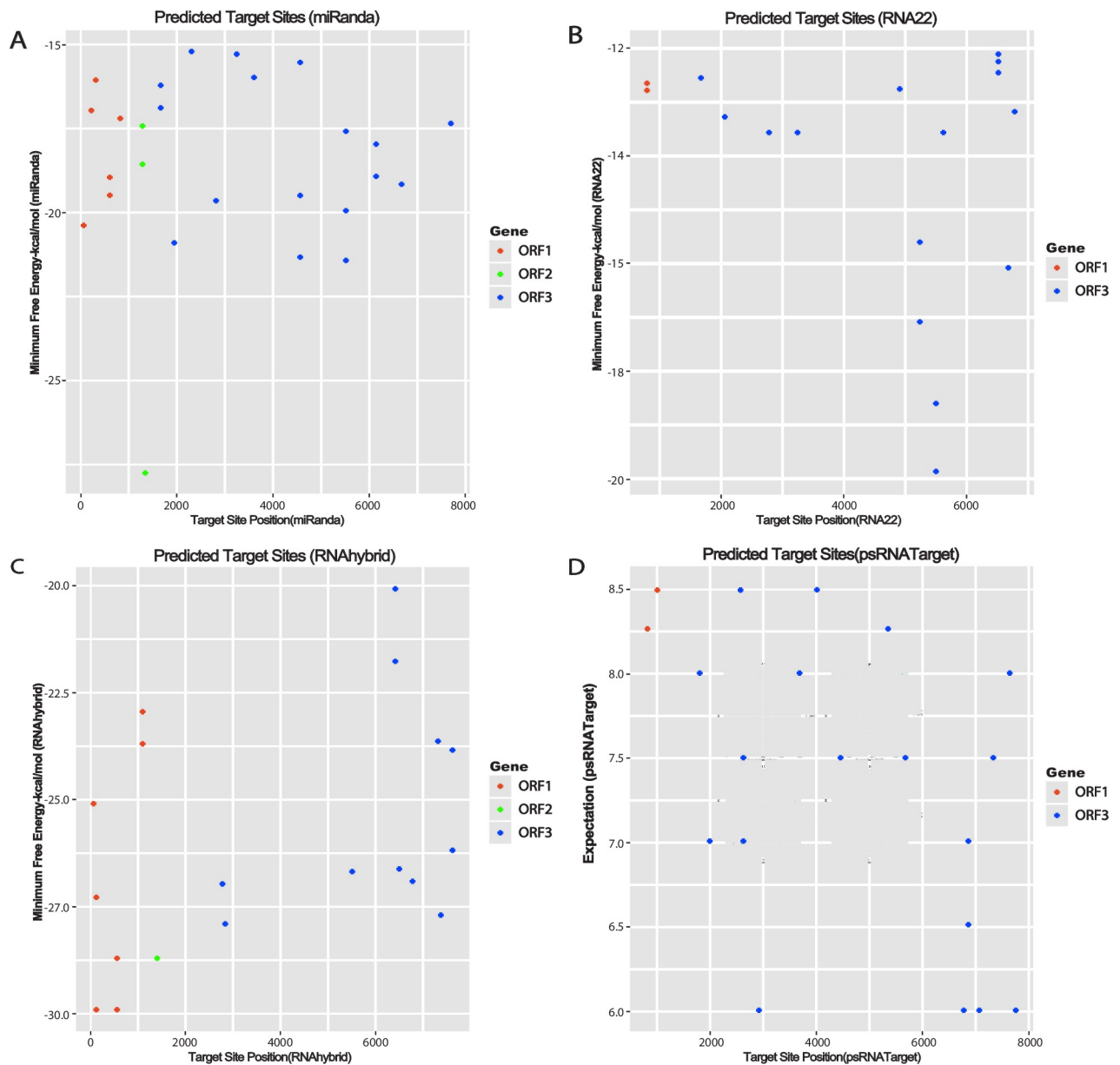


Fig 3. Target prediction of sugarcane miRNAs in the SCBV genome. Computational prediction of candidate miRNA targets in the genome of the SCBV. (A) miRNA targets obtained from miRanda. (B) RNA22 predicted potential hybridization sites. (C) Target sites of sugarcane miRNAs as identified by RNAhybrid. (D) Prediction results of target sites of sugarcane miRNAs as obtained by psRNATarget.

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target ORF3, including sof-miR159 (a, b, c, d, and e) at common locus 5534, sof-miR167 (a, b) at locus 2273, sof-miR168b at locus 4588, sof-miR408 (a, b, c, d, and e) at the two common loci of 4595 and 6695, ssp-miR166 at locus 1986, ssp-miR827 at locus 2816, and ssp-miR444 (a, b, and c-3p) at common locus 6184. Multiple loci interactions were predicted for the sof-miR159, sof-miR408, and ssp-miR444 families at nucleotide positions (5534–5552, 5576–5596), (4595–4615, 6695–6715) and (1679–1701, 3293–3313) of ORF3, respectively (Fig 3A).

Potential target binding sites were determined for ORF3 of the SCBV genome by the RNA22 algorithm. These included sof-miR168a at locus 3263, sof-miR168b at nucleotide positions 1693 and 3263, sof-miR396 at locus 2050, and ssp-827 at locus 2796 (Fig 3B). Multiple

loci interactions were also identified for the sof-miR159, sof-miR408, and ssp-miR444 families at nucleotide positions (5532, 6536), (5645, 6695), and (5246, 6793) respectively (Fig 3B). Suitable miRNAs that potentially targeted ORF3 were hybridized in order to understand the miRNA—mRNA interaction via RNAhybrid. As a result of, sof-miR159 (a, b, d and e) was detected at common locus 5535, along with sof-miR159c at locus 6518, sof-miR167 (a, b) at locus 2826, sof-miR169 at locus 7362, ssp-miR473 (a, b, c) at common locus 6438, ssp-miR444 (a, b) at locus 6796, ssp-miR444 c-3p at locus 2899 and ssp-miR1432 at locus 7314 (Fig 3C). ORF3 was targeted by several candidate miRNAs, including sof-miR159e at locus 2647, sof-miR396 at locus 5363, ssp-miR166 at locus 1986, ssp-miR437 (a, c) at locus 2647, ssp-miR827 at locus 7337, and ssp-miR444 (a, b and c-3p) at locus 6797, as identified by psRNATarget. Multiple loci interactions were observed for the sof-miR408 and ssp-miR444 families at the nucleotide positions of (1766–1786, 3669–3689, 5683–5702) and (4466–4486, 6797–6816, 6865–6885, 7079–7099), respectively (Fig 3D). The union plot indicates entire genome binding sites identified by the candidate miRNAs using target prediction tools (Fig 4, and S2 Table in S1 File).

3.5. Visualization and analysis of miRNA-target interaction network

Initially, the Circos plotting tool was designed to analyze mutations with comparative metagenomics and transcriptomic biological data [46]. To study a comprehensive visualization of host–virus interaction, we created a Circos plot to integrate biological data from sugarcane miRNAs and their predicted SCBV genomic target genes (ORFs) (Fig 5). In order to reduce visual graphical complexity and permit improved readability, we only used selected sugarcane miRNAs and their SCBV targets obtained from miRanda analysis. The miRanda algorithm considers seed-based interactions and the conservation level [47, 48]. The results suggest that biological data visualization of candidate miRNAs from sugarcane, with SCBV-encoded ORFs determines credible information of desirable preferred targets of SCBV ORFs using consensual miRNAs. We have combined sugarcane miRNA data and their predicted SCBV targets simultaneously in this manner.

3.6. Predicting common sugarcane miRNAs

Based on predicted targeting miRNAs from sugarcane to silence the SCBV genome, fourteen miRNAs (sof-miR156, sof-miR159c, sof-miR159e, sof-miR168a, sof-miR396, sof-miR408a, sof-miR408b, sof-miR408c, sof-miR408d, sof-miR408e, ssp-miR827, ssp-miR444a, ssp-miR444b and sof-miR444c-3p) were detected by union of consensus between the multiple algorithms (miRanda, RNA22, RNAhybrid and psRNATarget) used in this study (Fig 6). Moreover, SCBV genomic components (ORF1, ORF2, ORF3, and the large intergenic region (LIR)) were observed to be targeted by a total of eleven sugarcane miRNAs which were hybridized at unique positions within ORF1 (sof-miR156 (locus 818) and sof-miR168 (a, b) (locus 617)) ORF2 (ssp-miR166 (locus 1450), ORF3 (sof-miR159c (locus 5534) and sof-miR408 (a, b, c, d and e) (locus 6695), and the LIR sof-miR396 (locus 79)) according to intersection between two consensual algorithms (Table 2, and S3 Table in S1 File).

3.7. Predicting consensual sugarcane miRNAs for silencing the SCBV genome

Out of 28 sugarcane miRNAs, only six sugarcane miRNA (sof-miR159 (a, b, d and e) at common locus position 5535 and ssp-miR444 (a, b) at locus 6797) were predicted at the common locus by at least three of the algorithms used (Fig 7 and Table 2). Out of 14 consensual miRNAs, only one miRNA of *S. officinarum* (sof-miR159e at locus 5535), with a MFE of -26.7

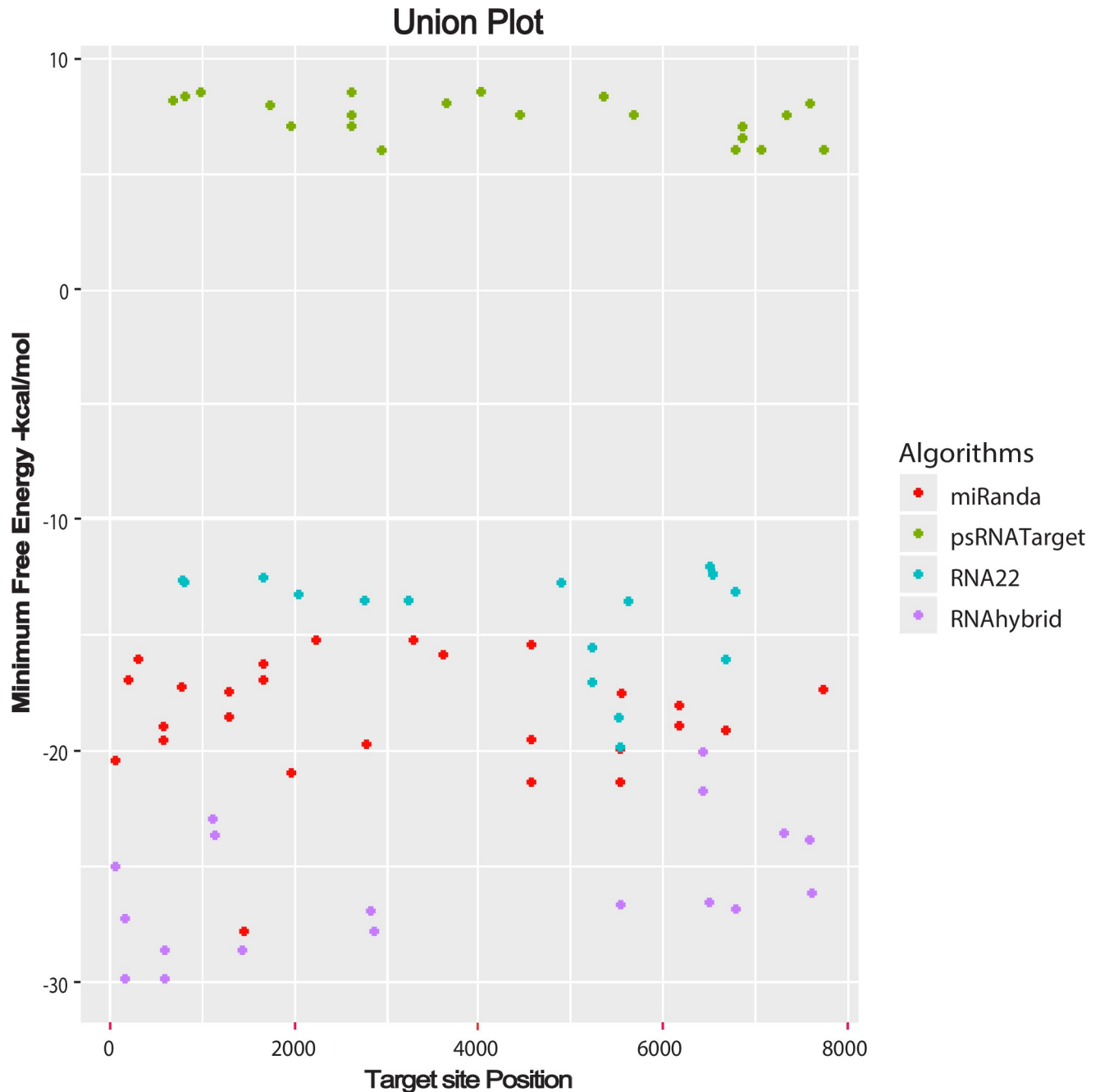


Fig 4. Union plot representing all the predicted sugarcane miRNA targets in the SCBV genome. miRNA target candidate prediction is represented as a union from all the algorithms used in this study.

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Kcal/mol, was considered as the top effective candidate in terms of support more efficient silencing of the SCBV genome. The efficacy of the sof-miR159e target against SCBV was validated by the suppression of RNAi-mediated viral combat through the cleavage of viral mRNA or translational inhibition [43]. Multiple loci interactions were observed for sof-miR159e at nucleotide positions 5534–5552 (consensus of three algorithms, namely, miRanda, RNA22, and RNAhybrid) and 2647 (psRNATarget) of ORF3.

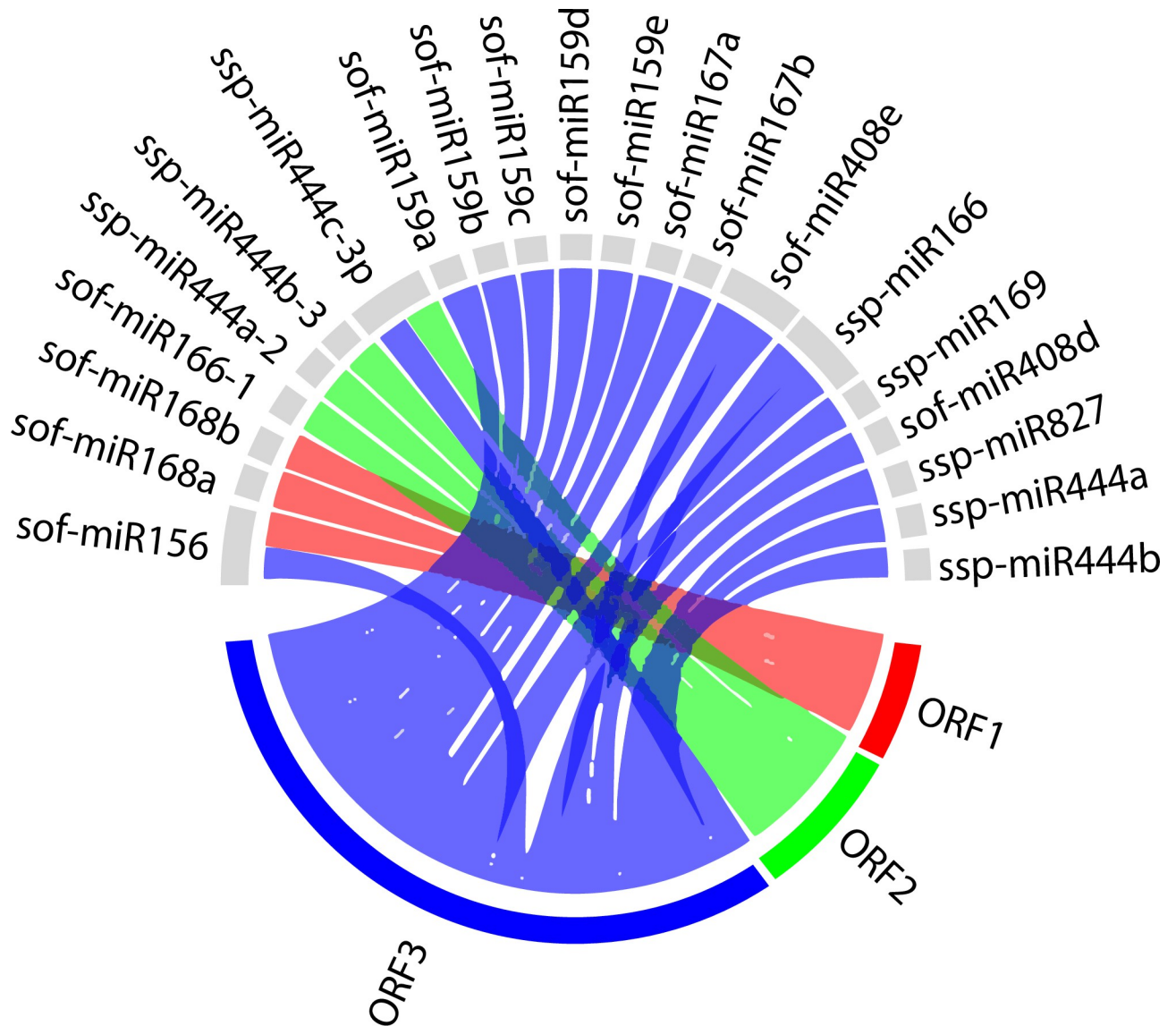


Fig 5. Circos plot representing miRNA-target interaction. Circos plot of genomic regulatory network interaction as predicted to be targeted by the sugarcane miRNAs. The red, green, and blue colored lines represent SCBV genome components (ORFs). The synergetic counterparts of sugarcane miRNAs and their target genes (ORFs) of the SCBV genome are interconnected with colored lines.

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3.8. Prediction of consensus secondary structures

The validation of consensual sugarcane miRNAs was confirmed by the prediction of their stable secondary structures using the RNAfold algorithm. Precursors of mature sugarcane miRNAs were manually curated. The MFE is the key factor to determine the stable secondary structures of precursors. All the predicted consensual sugarcane miRNA precursors were observed to possess lower MFE values (ranging from -57.70 to -114.70 kcal/mol) (Table 3).

The predicted secondary structures of six precursors of pre-miRNAs are shown in (Fig 8), as predicted by the intersection of three consensual algorithms at the same locus. The top stable secondary structure of the sof-MIR159e precursor was predicted with standard features (MFE: 107.50 Kcal/mol, MFEI: 1.06 Kcal/mol). The predicted secondary structures of 14 consensual sugarcane miRNAs passed the aforementioned standard criteria. We have determined

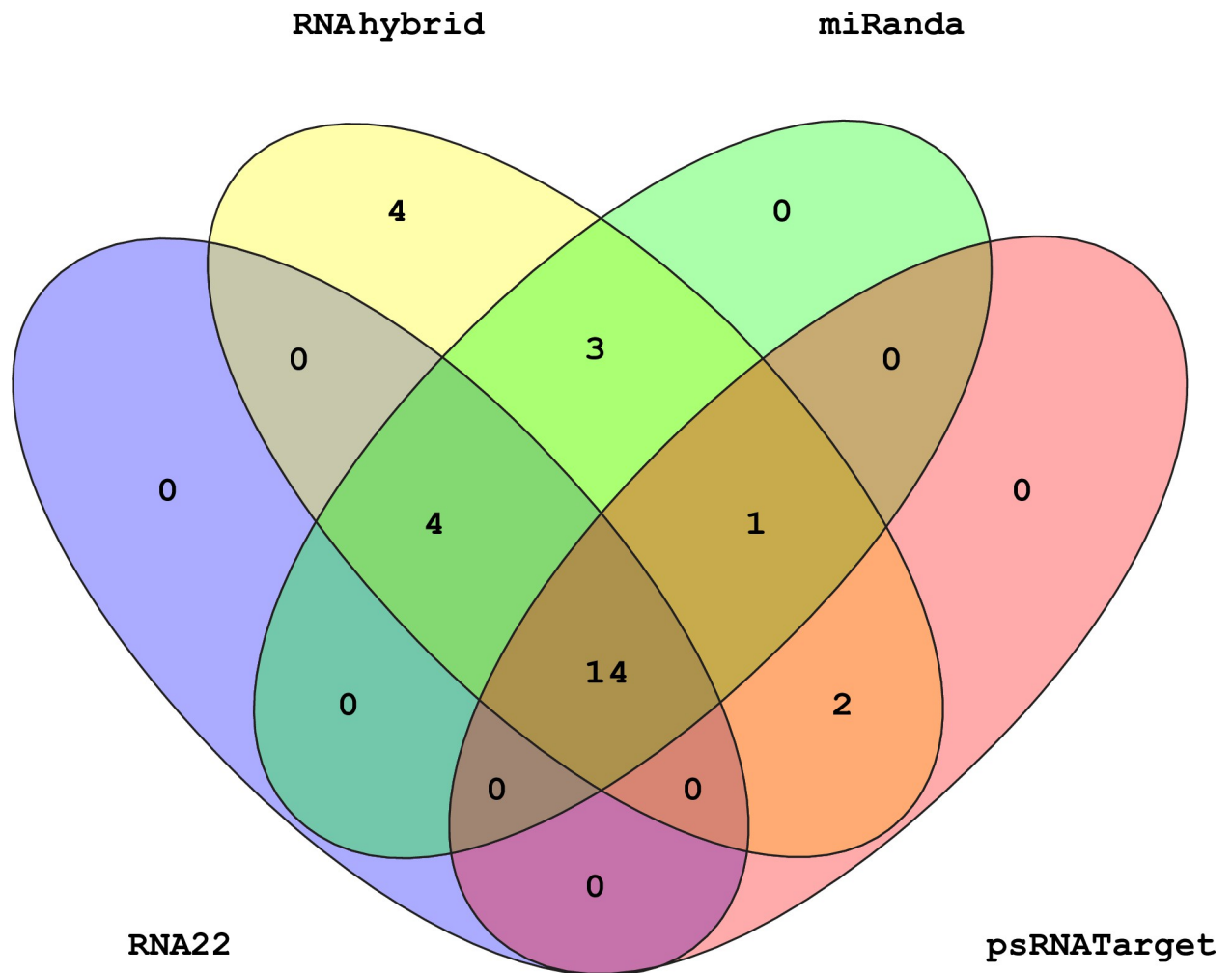


Fig 6. Venn diagram plot of SCBV genome targeted by sugarcane miRNAs. Venn diagram plot of the SCBV genome targeted by sugarcane miRNAs. In total, 28 loci are targeted by sugarcane miRNAs as predicted from four unique algorithms.

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the salient characteristics of six consensus precursor miRNAs in this study, such as the MFE, AMFE, MFEI, length precursor, and GC contents. In our studies, the length precursor ranges from 105–266 nucleotides, along with a MFE of -57.70 to -110.70 kcal/mol, AMFE of -39.92 to 60.09, GC content of 38–47%, and MFEI from -0.83 to -1.26.

3.9. Assessment of free energy (ΔG) of miRNA-mRNA interaction

The predicted consensual sugarcane miRNAs were validated by estimating the free energies of miRNA/target duplexes (Table 3). The free energies (ΔG) of six consensual sugarcane miRNAs were estimated as follows: sof-miR159 (a, b, d) (ΔG : -20.10 kcal/mol), sof-miR15e (ΔG : -20.40 kcal/mol), and ssp-miR444 (a, b) (ΔG : -14.50 kcal/mol).

3.10. Tissue preferential expression analysis of sugarcane miRNAs

We used the “PmiRExAt” database to search for the expression analysis of the predicted sugarcane miRNAs. Homologous miRNAs were present in all three plant species, i.e., maize, rice, and wheat (S1–S3 Figs). The expression of these microRNAs was identified in all tissue types

Table 2. Sugarcane miRNAs and their target positions in SCBV as identified by various algorithms.

Sugarcane miRNAs	Position miRanda	Position RNA22	Position RNAhybrid	Position psRNATarget	MFE* miRanda	MFE** RNA22	MFE RNAhybrid	Expectation psRNATarget
sof-miR156	818	817	7608	7609	-17.23	-12.7	-23.9	8
sof-miR159a	5534	5532	5535		-21.45	-19.9	-26.7	
sof-miR159a(1)	5576	6536			-17.54	-12.5		
sof-miR159b	5534	5532	5535		-21.45	-19.9	-26.7	
sof-miR159b(1)	5576	6536			-17.54	-12.5		
sof-miR159c	5534	5532	6518	1003	-20.02		-28	6
sof-miR159c(1)		6533				-12.1		
sof-miR159d	5534	5532	5535		-21.45	-19.9	-26.7	
sof-miR159d(1)	5576	6536			-17.54	-12.5		
sof-miR159e	5534	5532	5535		-21.45	-19.9	-26.7	
sof-miR159e(1)	3633	6536			-16	-12.1		
sof-miR167a	2273		2826		-15.24		-27	
sof-miR167b	2273		2826		-15.24		-27	
sof-miR168a	617	834	612	4046	-19.53	-12.8	-29.9	8.5
sof-miR168a(1)		3263				-13.6		
sof-miR168b	617	1693	612		-19	-26.7	-28.7	
sof-miR168b(1)	4588	4907			-15.49	-12.8		
sof-miR396	79	2050	79	5563	-20.44	-13.3	-25.1	8.25
sof-miR408a	6695	6695	174	3669	-19.19	-16.1	-27.3	8
sof-miR408a(1)	4595	5645		1766	-21.35	-13.6		8
sof-miR408b	6695	6695	174	3669	-19.19	-16.1	-27.3	8
sof-miR408b(1)	4595	5645		1766	-21.35	-13.6		8
sof-miR408c	6695	6695	174	3669	-19.19	-16.1	-27.3	8
sof-miR408c(1)	4595	5645		1766	-21.35	-13.6		8
sof-miR408d	6695	6695	174	3669	-19.19	-16.1	-27.3	8
sof-miR408d(1)	4595	5645		1766	-21.35	-13.6		8
sof-miR408e	6695	6695	174	5683	-19.19	-16.1	-29.9	7.5
sof-miR408e (1)	4595	5645		1766	-19.53	-13.6		8
sof-miR408e (2)	242				-17.01			
ssp-miR166	1449		1450	7750	-27.85		-28.7	6
ssp-miR166(1)	1986			1986	-20.95			7
ssp-miR169	7748		7362		-17.38		-27.7	
ssp-miR437a			6438	2646			-20.1	7
ssp-miR437b			6438				-20.1	
ssp-miR437c			6437	2647			-21.8	7.5
ssp-miR437c(1)				2974				6
ssp-miR528			7619				-26.2	
ssp-miR827	2816	2796	1170	7337	-19.73	-13.6	-23.7	7.5
ssp-miR444a	6184	6793	6796	6797	-18.04	-13.2	-26.9	6
ssp-miR444a(1)	3293	5246		6865	-15.23	-15.6		6.5
ssp-miR444a(2)	1301				-17.44			
ssp-miR444b	6184	6793	6796	6797	-18.04	-13.2	-26.9	6
ssp-miR444b(1)	3293	5246		7079	-15.23	-15.6		6
ssp-miR444b(2)	1301			4466	-17.44			7.5
ssp-miR444b(3)	1676				-16.25			
ssp-miR444c-3p	6184	5246	2899	6797	-18.95	-17.1	-27.9	6

(Continued)

Table 2. (Continued)

Sugarcane miRNAs	Position miRanda	Position RNA22	Position RNAhybrid	Position psRNATarget	MFE* miRanda	MFE** RNA22	MFE RNAhybrid	Expectation psRNATarget
ssp-miR444c-3p (1)	328			6865		-16.8		7
ssp-miR444c-3p (2)	1301					-18.59		
ssp-miR444c-3p (3)	1680					-16.96		
ssp-miR1128			1137				-23	
ssp-miR1432			7314				-23.6	

*MFE: Minimum free energy measured in /Kcal/mol where *MFE represents minimum folding energy measured in Kcal/mol.

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in each species. Therefore, the expression of sugarcane miRNAs was confirmed in other plant species, i.e., maize, rice, and wheat. Evidence of the existence of the same miRNAs in sugarcane is also provided. Most of the stated miRNAs have also been confirmed, in multiple studies, for their expression and roles in plant cellular pathways [49, 50].

4. Discussion

For the filtering of false positive results, we studied the effectiveness of the computational algorithms considered here to validate the miRNA target prediction data. We designed an effective approach for the validation of miRNA target prediction results at individual, union, and intersection levels. Computational prediction algorithms offer rapid methods to identify potential host-derived miRNA targets in virus genomes. Default parameters represent optimized specifications for each miRNA to its respective target site in the viral genome. This varies with respect to each algorithm/tool and can be modified for fine-tuning the settings or increasing the level of sensitivity for predicted sites. Default parameters are effective for screening out false-positive attachment sites for miRNAs using multiple prediction tools. miRanda is a widely used algorithm that includes the main aspects of miRNA–target prediction, such as the conservation level and miRNA 3'UTR site [51]. The RNA22 algorithm is a novel alternative option for exploring new miRNA–mRNA interactions because of its unique capabilities—although it has a high likelihood of generating false-positive results [47]. We calculated the MFE and determined the target inhibition as recommended by Brodersen by using RNAhybrid [37].

Several potential sugarcane miRNA targets and miRNA–mRNA interactions could be consensually predicted by all of the algorithms (Fig 7). Plant miRNAs are responsible for inducing the degradation of the target genes using perfect or imperfect complementarity base pairing [52]. The current study demonstrates that SCBV genome components (ORF1, ORF2, and ORF3) are susceptible to targeting by a set of consensual sugarcane miRNAs. In addition, sof-miR159 (a, b, d, and e) was found to target ORF3 at a consensual hybridization site by at least three algorithms (Fig 8). Free energy assessment is a dynamic feature of miRNA and target binding. Previous studies have revealed a significant correlation of free energy between the translational repression and the hybridization binding of the seed region [53]. The thermodynamic stability of the miRNA–mRNA duplex was estimated by the assessment of free energy to monitor site accessibility for the determination of the secondary structure duplex [27]. In order to validate miRNA–mRNA interaction, the free energy of a duplex was assessed (Table 2). Our prediction results show high stability for the sugarcane-encoded miRNA–

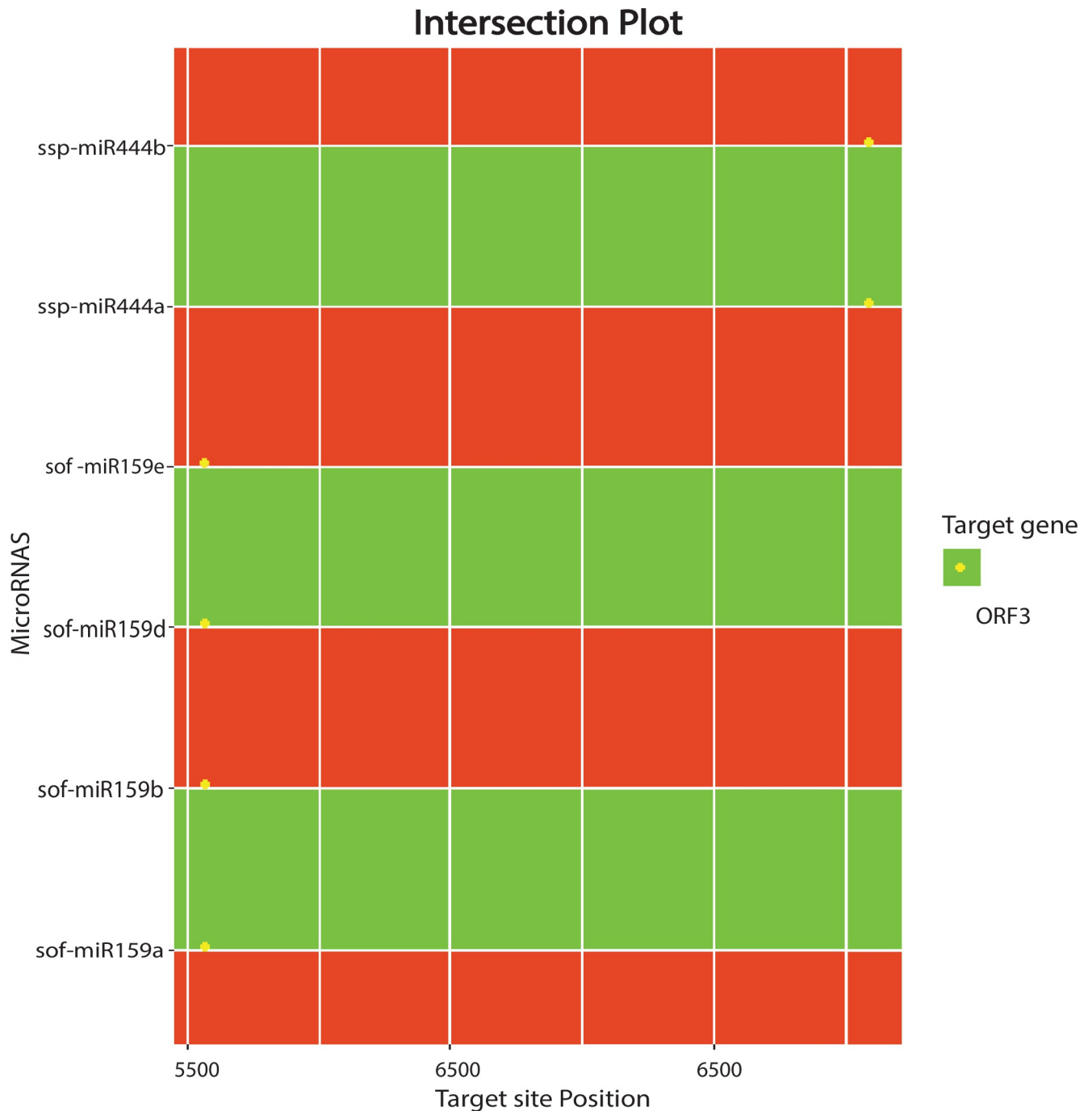


Fig 7. Intersection plot of sugarcane miRNAs predicted from at least three algorithms. The intersection plot was created with the miRNAs predicted from at least three algorithms (miRanda, RNA22 and RNAhybrid). Color codes given within the figure.

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SCBV-mRNA duplex at a low free energy level (Table 3 and Fig 8). The RNA duplex is considered to be more stable due to the stronger binding of miRNA to mRNA [54, 55].

We used union and intersection approaches to reduce false positive prediction. Union approaches rely on combining more than one target prediction tool when finding true and false targets. The sensitivity level for a predicted target increases due to a decrease in specificity.

Table 3. The salient parameters of precursor miRNAs were determined along with the estimation of free energy.

miRNA ID	Length miRNA	Length precursor	MFE ¹ (Kcal/mol)	AMFE ²	MFEI ³	(G+C)%	ΔG^4 (Kcal/mol)
sof-miR156	20	137	-66.20	-48.32	-0.96	50.00	-14.30
sof-miR159a	21	265	-110.30	-41.62	-0.87	47.60	-20.10
sof-miR159b	21	266	-110.30	-41.46	-0.87	47.60	-20.10
sof-miR159c	21	238	-110.60	-46.47	-0.88	52.38	-19.70
sof-miR159d	21	265	-105.80	-39.92	-0.83	47.60	-20.10
sof-miR159e	21	264	-107.50	-40.71	-1.06	38.09	-20.40
sof-miR168a	21	104	-66.20	-63.65	-1.02	61.90	-18.20
sof-miR396	21	134	-67.40	-50.29	-1.17	42.85	-19.60
sof-miR408a	21	283	-114.70	-40.53	-0.60	66.66	-16.00
sof-miR408b	21	286	-113.20	-39.58	-0.59	66.66	-16.00
sof-miR408c	21	286	-115.80	-40.48	-0.60	66.66	-16.00
sof-miR408d	21	215	-79.00	-36.76	-0.55	66.66	-16.00
sof-miR408e	21	283	-99.00	-34.98	-0.56	61.90	-16.00
ssp-miR827	21	130	-64.00	-49.23	-1.29	38.09	-17.90
ssp-miR444a	21	105	-57.70	-54.95	-1.15	47.62	-14.50
ssp-miR444b	21	106	-63.70	-60.09	-1.26	47.62	-14.50
ssp-miR444c	21	108	-61.80	-57.22	-1.33	42.85	-15.30

¹MFE is minimum free energy.

²AMFE represents adjusted minimum free energy.

³MFEI defines as minimum free energy index.

⁴ ΔG represents minimum free energy of duplex formation.

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An intersection approach is entirely different and depends upon the combination of two or more computational tools and enhances the specificity level of predicted targets due to a decrease in sensitivity [56]. Our target prediction results revealed that both computational approaches achieved the best outcomes with maximum performance for predicting and estimating the best targets (Figs 6 and 7). Previous studies have also reported the silencing of plant viruses using host-derived miRNAs when applying a set of computational algorithms. The identification and evaluation of best-fit candidate miRNA targets for different plants has been concluded successfully with potato virus Y (PVY) [57], maize chlorotic mottle virus (MCMV) [58], CLCuKoV-Bu [59], rice yellow mottle virus (RYMV), [60] and SCBGAV to find miRNA–target interaction [61]. We have designed an equal novel bioinformatics approach for target prediction in the SCBV genome to control the emerging presence of *Badnavirus* in sugarcane cultivars.

In our previous study, we identified the most ideal consensual sugarcane miRNA (sof-miR396) to target ORF3 of the SCBGAV genome using multiple computational algorithms [61]. The quantity of false positive miRNA–target interaction estimated by multiple algorithms depends upon the mode of miRNA–target recognition. MFE is also another important factor that affects miRNA–target interaction in result validation [62]. To set a lower MFE value will give rise to a higher probability of miRNA–target complex formation [63]. In the current study, for miRanda analysis, a stringent cut-off point of -15 kcal/mol was set for narrowing down the miRNA candidates. Similarly, to validate host–virus interaction, a MFE cut-off point of -20 kcal/mol applied for RNAhybrid analysis [32].

Although MFE has a considerable role for development of miRNA–mRNA complexes, it does not certify that interactions will lead to functional changes. In the current study, we have identified six potential miRNA hybridization binding sites that have exhibited low MFES and free energy for duplex formation. These predicted miRNAs not only have potential targets for

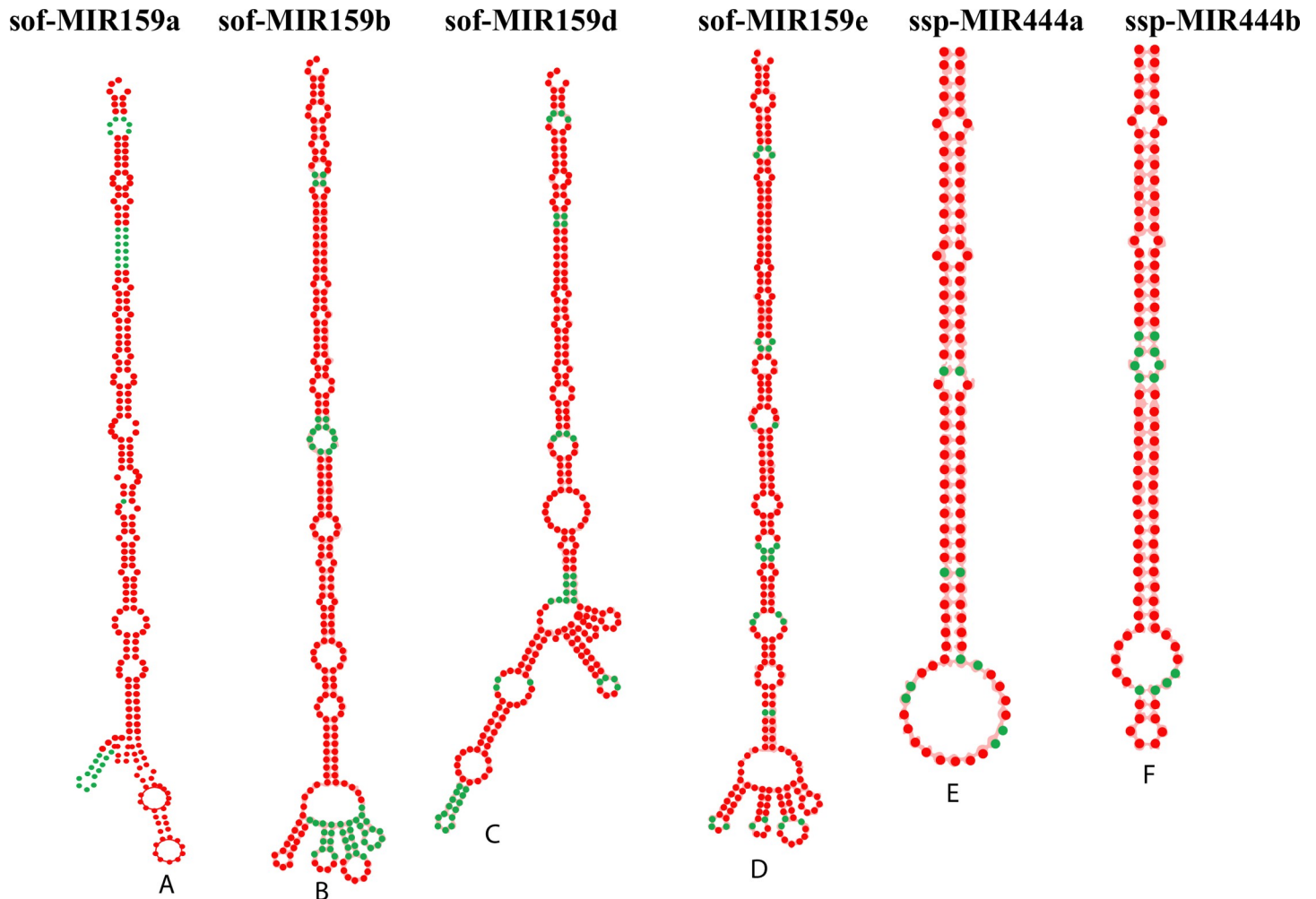


Fig 8. Prediction of secondary structures of stem-loop sequences of sugarcane miRNAs. Six pre-miRNA secondary structures (precursors of sugarcane miRNAs) were identified in this study by consensus between three algorithms. The sugarcane mature miRNA name IDs, accession IDs, MFEs and MFEIs are given as follows: (A) sof-MIR159a (MI0001756), -110.30 kcal/mol, -0.87; (B) sof-MIR159b (MI0001757), -110.30 kcal/mol, -0.87; (C) sof-MIR159d (MI0001758), -105.80 kcal/mol, -0.83; (D) sof-MIR159e (MI0001759), -107.50 kcal/mol, -1.06; (E) ssp-MIR444a (MI0018185), -57.70 kcal/mol, -1.15; (F) ssp-MIR444b (MI0018186), -63.70 kcal/mol, -1.26.

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the SCBV genome at the transgenic level but also have a stronger probability to develop miRNA–viral mRNA complex formation. These miRNAs also have chance to participate in a SCBV replication mechanism, where a consensus sugarcane miRNA (sof-miR396) has a binding site within the SCBV large intergenic region (LIR) at locus 79 as predicted by the miRanda and RNAhybrid algorithms. In the previous study, we predicted that sof-miR396 is an effective candidate to target the SCBGAV genome [61]. Notably, sof-miR159e was predicted by all the algorithms. Additionally, miR159 was explored and was found to present a strong role for silencing *GAMYB* to enable normal growth [64]. Phe-MIR159 involved in regulating the gene responsible for secondary thickening in *Phyllostachys edulis* [65]. It is important to assess the function of predicted potential consensual miRNAs for the identification of *Badnavirus* replication to demonstrate SCBV replication experimentally. A hypothetical model was designed to show that sugarcane-derived miRNAs can inhibit SCBV mRNA and sugarcane genes against SCBV virus (Fig 9). It facilitates plant-encoded miRNAs in the cleavage of SCBV miRNA.

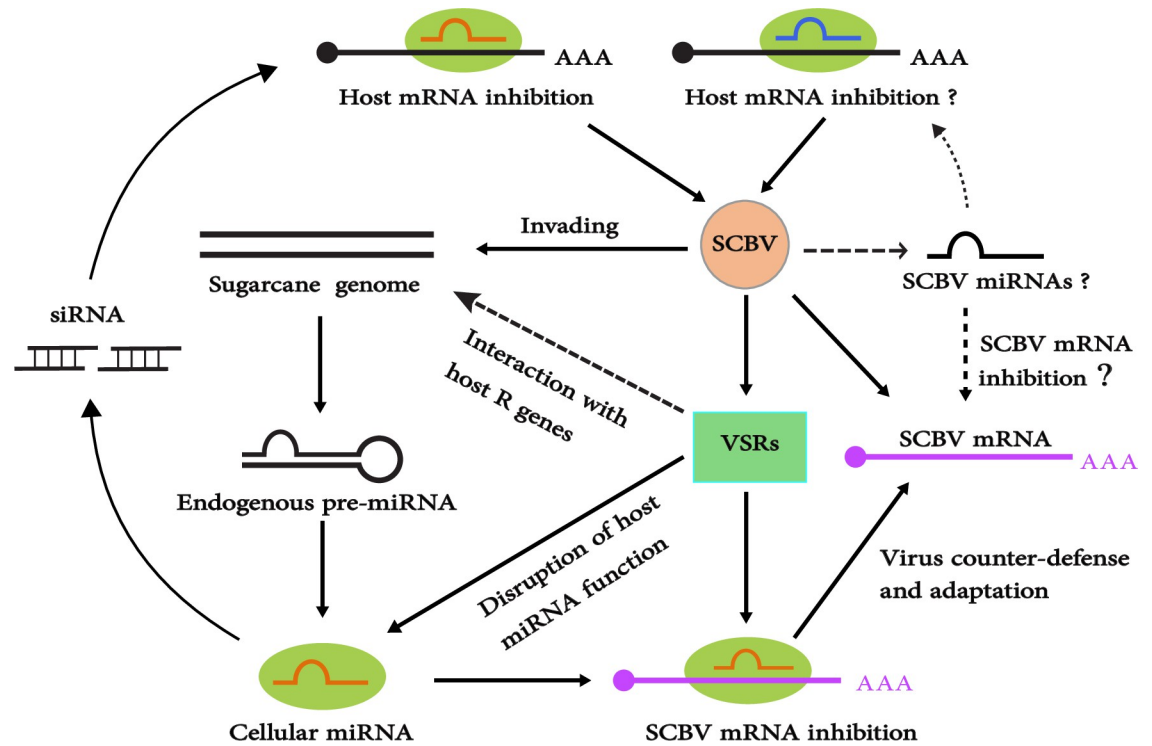


Fig 9. Schematic model designed for miRNA-mediated gene silencing in plant-virus interaction. SCBV can activate the production of endogenous sugarcane miRNAs post infection. Moreover, sugarcane miRNAs can target SCBV mRNA for degradation.

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RNAi screening is a novel technology for discovering various cellular functions and identifying host-derived factors of viruses [66]. Here, we selected 28 experimentally validated sugarcane miRNAs with annotated targets that are part of SCBV. amiRNA-based silencing technology has been successfully validated in many crop plants for controlling emerging plant viruses [23, 24, 26]. In summary, our computational work for SCBV genome silencing could offer a new approach for the production of antiviral agents. Furthermore, we demonstrated a method to minimize the novel antiviral effects of host-derived miRNAs against SCBV.

5. Conclusions

SCBV has appeared as a major problem in China. SCBV diminishes quantitative yields in all sugarcane cultivars. In the current study, prior to cloning, we have applied computational tools to predict and comprehensively analyze candidate miRNA from sugarcane against SCBV. Among them, sof-miR159e was predicted as the top effective candidate that could target the vital gene (ORF3) of the SCBV genome. Our results demonstrate an alternative strategy to existing molecular approaches that could be repurposed to control badnaviral infections. The current findings provide *in silico* evidence of a novel scheme to construct miRNA-mediated gene silencing therapeutics to combat SCBV.

Supporting information

S1 File.
(RAR)

S1 Fig. Tissue preferential expression heatmap of sugarcane miRNAs in maize.
(TIF)

S2 Fig. Tissue preferential expression heatmap of sugarcane miRNAs in rice.
(TIF)

S3 Fig. Tissue preferential expression heatmap of sugarcane miRNAs in wheat.
(TIF)

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References

1. Bouhida M., Lockhart B., and Olszewski N.E., An analysis of the complete sequence of a sugarcane bacilliform virus genome infectious to banana and rice. *Journal of General Virology*, 1993. 74(1): p. 15–22. <https://doi.org/10.1099/0022-1317-74-1-15> PMID: 8423447
2. Sun S.-R., et al., Molecular characterization of two divergent variants of sugarcane bacilliform viruses infecting sugarcane in China. *European Journal of Plant Pathology*, 2016. 145(2): p. 375–384.
3. King A.M., et al., *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*. Vol. 9. 2011: Elsevier.
4. Geijskes R.J., et al., Sequence analysis of an Australian isolate of sugarcane bacilliform badnavirus. *Archives of virology*, 2002. 147(12): p. 2393–2404. <https://doi.org/10.1007/s00705-002-0879-2> PMID: 12491105
5. Muller E., et al., High molecular variability of sugarcane bacilliform viruses in Guadeloupe implying the existence of at least three new species. *Virus research*, 2011. 160(1–2): p. 414–419. <https://doi.org/10.1016/j.virusres.2011.06.016> PMID: 21741419

6. Ahmad K., et al., Presence of diverse sugarcane bacilliform viruses infecting sugarcane in China revealed by pairwise sequence comparisons and phylogenetic analysis. *The plant pathology journal*, 2019. 35(1): p. 41. <https://doi.org/10.5423/PPJ.OA.08.2018.0156> PMID: 30828278
7. Bousalem M., Douzery E.J., and Seal S., Taxonomy, molecular phylogeny and evolution of plant reverse transcribing viruses (family Caulimoviridae) inferred from full-length genome and reverse transcriptase sequences. *Archives of virology*, 2008. 153(6): p. 1085–1102. <https://doi.org/10.1007/s00705-008-0095-9> PMID: 18483693
8. Autrey L., et al. The distribution of sugarcane bacilliform virus in various geographical regions. in *Proc Int Soc Sugar Cane Technol*. 1995.
9. Lockhart, B., M. Irey, and J. Comstock. Sugarcane bacilliform virus, sugarcane mild mosaic virus and sugarcane yellow leaf syndrome. in *Sugarcane germplasm conservation and exchange: report of an international workshop held in Brisbane, Queensland, Australia, 28–30 June 1995*. 1996. Canberra, Australia: Australian Centre for International Agricultural . . .
10. Ruiz-Ferrer V. and Voinnet O., Roles of plant small RNAs in biotic stress responses. *Annual review of plant biology*, 2009. 60: p. 485–510. <https://doi.org/10.1146/annurev.arplant.043008.092111> PMID: 19519217
11. Hamilton A.J. and Baulcombe D.C., A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science*, 1999. 286(5441): p. 950–952. <https://doi.org/10.1126/science.286.5441.950> PMID: 10542148
12. Baulcombe D., RNA silencing in plants. *Nature*, 2004. 431(7006): p. 356–363. <https://doi.org/10.1038/nature02874> PMID: 15372043
13. Ketting R.F., The many faces of RNAi. *Developmental cell*, 2011. 20(2): p. 148–161. <https://doi.org/10.1016/j.devcel.2011.01.012> PMID: 21316584
14. Muhammad T., et al., RNA interference: a natural immune system of plants to counteract biotic stressors. *Cells*, 2019. 8(1): p. 38. <https://doi.org/10.3390/cells8010038> PMID: 30634662
15. Khvorova A., Reynolds A., and Jayasena S.D., Functional siRNAs and miRNAs exhibit strand bias. *Cell*, 2003. 115(2): p. 209–216. [https://doi.org/10.1016/s0092-8674\(03\)00801-8](https://doi.org/10.1016/s0092-8674(03)00801-8) PMID: 14567918
16. Wang W. and Galili G., Tuning the Orchestra: miRNAs in plant immunity. *Trends in plant science*, 2019. 24(3): p. 189–191. <https://doi.org/10.1016/j.tplants.2019.01.009> PMID: 30732937
17. Liu S.-R., et al., MicroRNA-mediated gene silencing in plant defense and viral counter-defense. *Frontiers in microbiology*, 2017. 8: p. 1801. <https://doi.org/10.3389/fmicb.2017.01801> PMID: 28979248
18. Gaffar F.Y. and Koch A., Catch me if you can! RNA silencing-based improvement of antiviral plant immunity. *Viruses*, 2019. 11(7): p. 673. <https://doi.org/10.3390/v11070673> PMID: 31340474
19. Qu J., Ye J., and Fang R., Artificial microRNA-mediated virus resistance in plants. *Journal of virology*, 2007. 81(12): p. 6690–6699. <https://doi.org/10.1128/JVI.02457-06> PMID: 17344304
20. Rosa C., et al., RNA interference mechanisms and applications in plant pathology. *Annual review of phytopathology*, 2018. 56: p. 581–610. <https://doi.org/10.1146/annurev-phyto-080417-050044> PMID: 29979927
21. Schwab R., et al., Highly specific gene silencing by artificial microRNAs in Arabidopsis. *The Plant Cell*, 2006. 18(5): p. 1121–1133. <https://doi.org/10.1105/tpc.105.039834> PMID: 16531494
22. Ossowski S., Schwab R., and Weigel D., Gene silencing in plants using artificial microRNAs and other small RNAs. *The Plant Journal*, 2008. 53(4): p. 674–690. <https://doi.org/10.1111/j.1365-313X.2007.03328.x> PMID: 18269576
23. Niu Q.-W., et al., Expression of artificial microRNAs in transgenic Arabidopsis thaliana confers virus resistance. *Nature biotechnology*, 2006. 24(11): p. 1420–1428. <https://doi.org/10.1038/nbt1255> PMID: 17057702
24. Ali I., et al., Artificial microRNA-mediated resistance against the monopartite begomovirus Cotton leaf curl Burewala virus. *Virology journal*, 2013. 10(1): p. 231. <https://doi.org/10.1186/1743-422X-10-231> PMID: 23844988
25. Zhang X., et al., Expression of artificial microRNAs in tomato confers efficient and stable virus resistance in a cell-autonomous manner. *Transgenic research*, 2011. 20(3): p. 569–581. <https://doi.org/10.1007/s11248-010-9440-3> PMID: 20835923
26. Petchthai U., Le Yee C.S., and Wong S.-M., Resistance to CymMV and ORSV in artificial microRNA transgenic *Nicotiana benthamiana* plants. *Scientific reports*, 2018. 8(1): p. 1–8. <https://doi.org/10.1038/s41598-017-17765-5> PMID: 29311619
27. Peterson S.M., et al., Common features of microRNA target prediction tools. *Frontiers in genetics*, 2014. 5: p. 23. <https://doi.org/10.3389/fgene.2014.00023> PMID: 24600468

28. Kozomara A., Birgaoanu M., and Griffiths-Jones S., miRBase: from microRNA sequences to function. *Nucleic acids research*, 2019. 47(D1): p. D155–D162. <https://doi.org/10.1093/nar/gky1141> PMID: 30423142
29. Griffiths-Jones S., et al., miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic acids research*, 2006. 34(suppl_1): p. D140–D144. <https://doi.org/10.1093/nar/gkj112> PMID: 16381832
30. Kozomara A. and Griffiths-Jones S., miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic acids research*, 2014. 42(D1): p. D68–D73. <https://doi.org/10.1093/nar/gkt1181> PMID: 24275495
31. Karuppaiah R., Viswanathan R., and Kumar V.G., Genetic diversity of Sugarcane bacilliform virus isolates infecting *Saccharum* spp. in India. *Virus genes*, 2013. 46(3): p. 505–516. <https://doi.org/10.1007/s11262-013-0890-6> PMID: 23430710
32. Enright A.J., et al., MicroRNA targets in *Drosophila*. *Genome biology*, 2003. 5(1): p. R1. <https://doi.org/10.1186/gb-2003-5-1-r1> PMID: 14709173
33. John B., et al., Human microRNA targets. *PLoS Biol*, 2004. 2(11): p. e363. <https://doi.org/10.1371/journal.pbio.0020363> PMID: 15502875
34. Miranda K.C., et al., A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. *Cell*, 2006. 126(6): p. 1203–1217. <https://doi.org/10.1016/j.cell.2006.07.031> PMID: 16990141
35. Lohr P. and Rigoutsos I., Interactive exploration of RNA22 microRNA target predictions. *Bioinformatics*, 2012. 28(24): p. 3322–3323. <https://doi.org/10.1093/bioinformatics/bts615> PMID: 23074262
36. Krüger J. and Rehmsmeier M., RNAhybrid: microRNA target prediction easy, fast and flexible. *Nucleic acids research*, 2006. 34(suppl_2): p. W451–W454. <https://doi.org/10.1093/nar/gkl243> PMID: 16845047
37. Brodersen P., et al., Widespread translational inhibition by plant miRNAs and siRNAs. *Science*, 2008. 320(5880): p. 1185–1190. <https://doi.org/10.1126/science.1159151> PMID: 18483398
38. Dai X., Zhuang Z., and Zhao P.X., psRNATarget: a plant small RNA target analysis server (2017 release). *Nucleic acids research*, 2018. 46(W1): p. W49–W54. <https://doi.org/10.1093/nar/gky316> PMID: 29718424
39. Dai X. and Zhao P.X., psRNATarget: a plant small RNA target analysis server. *Nucleic acids research*, 2011. 39(suppl_2): p. W155–W159. <https://doi.org/10.1093/nar/gkr319> PMID: 21622958
40. Krzywinski M., et al., Circos: an information aesthetic for comparative genomics. *Genome research*, 2009. 19(9): p. 1639–1645. <https://doi.org/10.1101/gr.092759.109> PMID: 19541911
41. Lorenz R., et al., ViennaRNA Package 2.0. *Algorithms for molecular biology*, 2011. 6(1): p. 26. <https://doi.org/10.1186/1748-7188-6-26> PMID: 22115189
42. Bernhart S.H., et al., Partition function and base pairing probabilities of RNA heterodimers. *Algorithms for Molecular Biology*, 2006. 1(1): p. 3. <https://doi.org/10.1186/1748-7188-1-3> PMID: 16722605
43. Gurjar A.K.S., et al., PmiRExAt: plant miRNA expression atlas database and web applications. *Database*, 2016. 2016. <https://doi.org/10.1093/database/baw060> PMID: 27081157
44. Gandrud C., *Reproducible research with R and R studio*. 2013: CRC Press. <https://doi.org/10.1097/MPH.0b013e31829cdeba> PMID: 23823111
45. Jacquot E., et al., The open reading frame 2 product of cacao swollen shoot badnavirus is a nucleic acid-binding protein. *Virology*, 1996. 225(1): p. 191–195. <https://doi.org/10.1006/viro.1996.0587> PMID: 8918546
46. Naquin D., et al., CIRCUS: a package for Circos display of structural genome variations from paired-end and mate-pair sequencing data. *BMC bioinformatics*, 2014. 15(1): p. 1–6. <https://doi.org/10.1186/1471-2105-15-198> PMID: 24938393
47. Riffo-Campos Á.L., Riquelme I., and Brebi-Mieville P., Tools for sequence-based miRNA target prediction: what to choose? *International journal of molecular sciences*, 2016. 17(12): p. 1987. <https://doi.org/10.3390/ijms17121987> PMID: 27941681
48. Betel D., et al., Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome biology*, 2010. 11(8): p. 1–14. <https://doi.org/10.1186/gb-2010-11-8-r90> PMID: 20799968
49. Qiu L., et al., Integrated mRNA and small RNA sequencing reveals microRNA regulatory network associated with internode elongation in sugarcane (*Saccharum officinarum* L.). *BMC genomics*, 2019. 20(1): p. 1–14. <https://doi.org/10.1186/s12864-018-5379-1> PMID: 30606130

50. Su Y., et al., A dynamic degradome landscape on miRNAs and their predicted targets in sugarcane caused by *Sporisorium scitamineum* stress. *BMC genomics*, 2019. 20(1): p. 1–21. <https://doi.org/10.1186/s12864-018-5379-1> PMID: 30606130
51. Betel D., et al., Comprehensive modeling of microRNA targets predicts functional non-conserved and non-canonical sites. *Genome biology*, 2010. 11(8): p. R90. <https://doi.org/10.1186/gb-2010-11-8-r90> PMID: 20799968
52. Jones-Rhoades M.W., Bartel D.P., and Bartel B., MicroRNAs and their regulatory roles in plants. *Annu. Rev. Plant Biol.*, 2006. 57: p. 19–53. <https://doi.org/10.1146/annurev.arplant.57.032905.105218> PMID: 16669754
53. Doench J.G. and Sharp P.A., Specificity of microRNA target selection in translational repression. *Genes & development*, 2004. 18(5): p. 504–511. <https://doi.org/10.1101/gad.1184404> PMID: 15014042
54. Lewis B.P., Burge C.B., and Bartel D.P., Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *cell*, 2005. 120(1): p. 15–20. <https://doi.org/10.1016/j.cell.2004.12.035> PMID: 15652477
55. Huang Y., et al., A study of miRNAs targets prediction and experimental validation. *Protein & cell*, 2010. 1(11): p. 979–986. <https://doi.org/10.1007/s13238-010-0129-4> PMID: 21153515
56. M Witkos T., Koscianska E., and J Krzyzosiak W., Practical aspects of microRNA target prediction. *Current molecular medicine*, 2011. 11(2): p. 93–109. <https://doi.org/10.2174/156652411794859250> PMID: 21342132
57. Iqbal M.S., et al., Prediction of host-derived miRNAs with the potential to target PVY in potato plants. *Frontiers in Genetics*, 2016. 7: p. 159. <https://doi.org/10.3389/fgene.2016.00159> PMID: 27683585
58. Iqbal M.S., et al., In silico MCMV silencing concludes potential host-derived miRNAs in maize. *Frontiers in plant science*, 2017. 8: p. 372. <https://doi.org/10.3389/fpls.2017.00372> PMID: 28400775
59. Akhter Y. and Khan J.A., Genome wide identification of cotton (*Gossypium hirsutum*)-encoded microRNA targets against Cotton leaf curl Burewala virus. *Gene*, 2018. 638: p. 60–65. <https://doi.org/10.1016/j.gene.2017.09.061> PMID: 28964896
60. Jabbar B., et al., Target prediction of candidate miRNAs from *Oryza sativa* for silencing the RYMV genome. *Computational biology and chemistry*, 2019. 83: p. 107127. <https://doi.org/10.1016/j.compbiolchem.2019.107127> PMID: 31542706
61. Ashraf F., et al., A novel computational approach to the silencing of Sugarcane Bacilliform Guadeloupe A Virus determines potential host-derived MicroRNAs in sugarcane (*Saccharum officinarum* L.). *PeerJ*, 2020. 8: p. e8359. <https://doi.org/10.7717/peerj.8359> PMID: 31976180
62. Pinzón N., et al., microRNA target prediction programs predict many false positives. *Genome research*, 2017. 27(2): p. 234–245. <https://doi.org/10.1101/gr.205146.116> PMID: 28148562
63. Kertesz M., et al., The role of site accessibility in microRNA target recognition. *Nature genetics*, 2007. 39(10): p. 1278–1284. <https://doi.org/10.1038/ng2135> PMID: 17893677
64. Millar A.A., Lohe A., and Wong G., Biology and Function of miR159 in Plants. *Plants*, 2019. 8(8): p. 255. <https://doi.org/10.3390/plants8080255> PMID: 31366066
65. Cheng Z., et al., Integrated mRNA, MicroRNA Transcriptome and Degradome Analyses Provide Insights into Stamen Development in Moso Bamboo. *Plant and Cell Physiology*, 2020. 61(1): p. 76–87. <https://doi.org/10.1093/pcp/pcz179> PMID: 31550004
66. Hirsch A.J., The use of RNAi-based screens to identify host proteins involved in viral replication. *Future microbiology*, 2010. 5(2): p. 303–311. <https://doi.org/10.2217/fmb.09.121> PMID: 20143951