applied microbiology

MDPI

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In Silico Identification of Banana High-Confidence MicroRNA Binding Sites Targeting Banana Streak GF Virus

Muhammad Aleem Ashraf [1,2,*,†], Babar Ali [3,†], Maryam Fareed [4], Ahsan Sardar [4], Eisha Saeed [4], Samaa Islam [4], Shaher Bano [4] and Naitong Yu [1,*]

[1] Institute of Tropical Biosciences and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China
[2] Department of Biosciences and Technology, Faculty of Sciences, Emerson University, Multan 60000, Pakistan
[3] Department of Biosciences, COMSTAS University Islamabad, Park Road, Tarlai Kalan, Islamabad 45550, Pakistan; sp23-rbi-002@isbstudent.comsats.edu.pk
[4] Institute of Biological Sciences, Khwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan 64200, Pakistan; maryamfareed2027@gmail.com (M.F.); ahsansardar412@gmail.com (A.S.); eishasaeed05@gmail.com (E.S.); samaaislam25@gmail.com (S.I.); shaherbanoismail789@gmail.com (S.B.)
* Correspondence: aleem.ashraf@eum.edu.pk (M.A.A.); yunaitong@163.com (N.Y.)
† These authors contributed equally to this work.

Abstract: Banana streak GF virus (BSGFV) is the extremely dangerous monopartite badnavirus (genus, *Badnavirus*; family, *Caulimoviridae*) of banana (Musa acuminata AAA Group) that imposes a serious threat to global banana production. The BSGFV causes a devastating pandemic in banana crops, transmitted by deadly insect pest mealybug vectors and replicated through an RNA intermediate. The BSGFV is a reverse-transcribing DNA virus that has a monopartite open circular double-stranded DNA (dsDNA) genome with a length of 7325 bp. RNA interference (RNAi) is a natural mechanism that has revolutionized the target gene regulation of various organisms to combat virus infection. The current study aims to locate the potential target binding sites of banana-encoded microRNAs (mac-miRNAs) on the BSGFV-dsDNA-encoded mRNAs based on three algorithms, RNA22, RNAhybrid and TAPIR. Mature banana ($2n = 3x = 33$) miRNAs ($n = 32$) were selected and hybridized to the BSGFV genome (MN296502). Among the 32 targeted mature locus-derived mac-miRNAs investigated, two banana mac-miRNA homologs (mac-miR162a and mac-miR172b) were identified as promising naturally occurring biomolecules to have binding affinity at nucleotide positions 5502 and 9 of the BSGFV genome. The in silico banana-genome-encoded mac-miRNA/mbg-miRNA-regulatory network was developed with the BSGFV—ORFs using Circos software (version 0.69-9) to identify potential therapeutic target proteins. Therefore, the current work provides useful biological material and opens a new range of opportunities for generating BSGFV-resistant banana plants through the genetic manipulation of the selected miRNAs.

Keywords: *Badnavirus*; Banana streak GF virus; binding affinity; in silico tools; gene silencing; microRNA; RNAi; R-language; target prediction

Academic Editor: Ian Connerton

Received: 27 December 2024
Revised: 17 January 2025
Accepted: 24 January 2025
Published: 27 January 2025

Citation: Ashraf, M.A.; Ali, B.; Fareed, M.; Sardar, A.; Saeed, E.; Islam, S.; Bano, S.; Yu, N. In Silico Identification of Banana High-Confidence MicroRNA Binding Sites Targeting Banana Streak GF Virus. *Appl. Microbiol.* **2025**, *5*, 13. https://doi.org/10.3390/applmicrobiol5010013

1. Introduction

The "Cavendish" banana (Musa acuminata, AAA) is an economically important popular respiratory climacteric tropical, most widely consumed fresh fruit and appreciated around the worldwide [1]. "Cavendish" banana is a triploid (AAA) cultivar banana. The

"Cavendish" banana variety is developed by intraspecific hybridization in *Musa acuminata* (A-genome, $2n = 2x = 22$) [2]. Banana streak disease (BSD) is the most destructive disease of banana that threatens food security and is caused by viruses of the genus *Badnavirus* (*Caulimoviridae*) that are approved by the ICTV [3]. Badnaviruses are transmitted by the most prevalent sap-feeding mealybug species such as *Planococcus citri (Pseudococcidae: Hemiptera)* [4].

The Cavendish banana cultivars, most widely grown in China, lack resistance to banana streak badnavirus infection [5–7]. The banana streak GF virus (BSGFV) monopartite, non-enveloped, bacilliform, circular double-stranded (ds) DNA is 7325 bp in length [5]. The *Badnavirus* genome usually contains a minimum of three well-defined, computationally predicted open reading frames, referred to as ORFs 1–3 [8–10]. The ORF1 encodes a hypothetical protein. The ORFII encodes a DNA binding protein, and a polyprotein was encoded by ORFIII. The intergenic region (IR), which is separated by ORF3 and ORF1, contains cis-regulatory motifs. The polyprotein is cleaved by a virus-encoded aspartic protease to multiple functional subunits. There is capsid protein, movement protein, aspartate protease, reverse transcriptase and RNase H [5,9,11,12].

RNA interference (RNAi) is a powerful tool and reliable method for high-throughput gene functional screening in biology laboratories worldwide [13,14]. RNAi is a conserved intracellular process that is being increasingly utilized for crop protection [13]. MicroRNAs (miRNAs) are 18–24 nucleotide, endogenous, small single-stranded (ss), non-coding RNA biomolecules transcribed by RNA polymerase II, capped and polyadenylated using pre-miRNAs [15–19]. The miRNAs are involved to regulate host–virus interactions, and cell growth, and appear as key cytoplasmic regulators of gene expression [20,21]. Artificial microRNA (amiRNA) technology is based on the design, synthesis and characterization of endogenous miRNA precursor. The amiRNA has revolutionized as a highly effective, safe and co-friendly antiviral defense approach to develop resistance in crop plants against viral infection [22–26].

The in silico identification, annotation and expression analysis of mature miRNAs in banana has opened new ways to evaluate biotic and abiotic stresses [27–29]. Recently, 32 conserved mature banana mac-miRNAs were annotated, identified and further experimentally validated [30]. Bioinformatics technology accelerates to design amiRNA constructs based on banana-locus-derived miRNA-binding sites in the dsDNA-encoded genome of the BSGFV. In this study, current therapeutic strategies based on a computational approach were designed for combatting BSGFV infection in banana cultivars through in silico tools. The predicted banana miRNAs can lead to the construction of amiRNA-based expression cassettes for transformation in the banana plants.

2. Materials and Methods

2.1. Biological Data

In a previous study, 32 mature banana microRNAs, both *Musa acuminata*-microRNA (mac-miR156-mac-5538) and Musa ABB Group-microRNA (mbg-miR397-mbg-399), have been computationally predicted (Supplementary File S1) [30]. In our previous study, the full-length genomic transcript (7325 bases) of BSGFV-YN (accession No. MN296502) was identified, purified and characterized from infected banana (Cavendish Musa AAA group) [5]. The full-length BSGFV dsDNA genome sequence was downloaded from the (NCBI) database [31].

2.2. Target Prediction in BSGFV Genome

Reliable target predictions were based on publicly available "three computational algorithms" cited in the literature. RNA22, RNAhybrid and TAPIR were selected to analyze

banana-encoded miRNAs for targeting the BSGFV-dsDNA-encoded mRNAs. Analysis was carried out for the banana miRNA sequences and full-length genomic transcript sequence of BSGFV, processed in plain FASTA text format.

2.3. RNA22 Algorithm

The RNA22 relies on a web-based prediction algorithm that implements a non-seed-based pattern recognition approach for detecting and analyzing miRNA-binding target sites [32,33]. Default parameters were considered to find the binding strength of banana mature miRNAs in the BSGFV genome. The MFE was set as −15.00 Kcal/mol.

2.4. RNAhybrid Algorithm

The RNAhybrid (version 2.1.2) algorithm is an easy-to-use, online flexible tool. RNAhybrid is used to calculate the minimum hybridization energy using the MFE model (−20.00 Kcal/mol). Free energy calculation is an initial and key step for prediction [34].

2.5. TAPIR Algorithm

The TAPIR is a state-of-the art plant-based algorithm to identify seed-based miRNA–mRNA interaction. TAPIR is based on the MFE ratio in plants [35]. TAPIR was run with the standard (default) parameters.

2.6. RNAcofold Algorithm

RNAcofold is a newly develop web-based algorithm that was used to evaluate miRNA–mRNA interactions based on free-energy assessment [36].

2.7. Discovering Banana-Genome-Encoded miRNA-Target Interaction

The miRNA–target interaction was mapped between banana mature miRNAs and ORFs of the BSGFV genome using the Circos algorithm [37].

2.8. Graphical Representation

The predicted miRNA-binding sites were processed and interpreted to generate graphical representation using the open source software package R (version 3.1) [38].

2.9. BSGFV Genome Analysis

The dsDNA genome of the BSGFV was annotated to generate ORFs using the pDRW32 (AcaClone software) (v 1.1.152).

3. Results

3.1. Banana miRNA's Loci n BSGFV Genome

The presented multiple computational framework combines biological data and miRNA prediction algorithms to detect banana-encoded miRNAs with potential to target the BSGFV dsDNA genome (Figure 1).

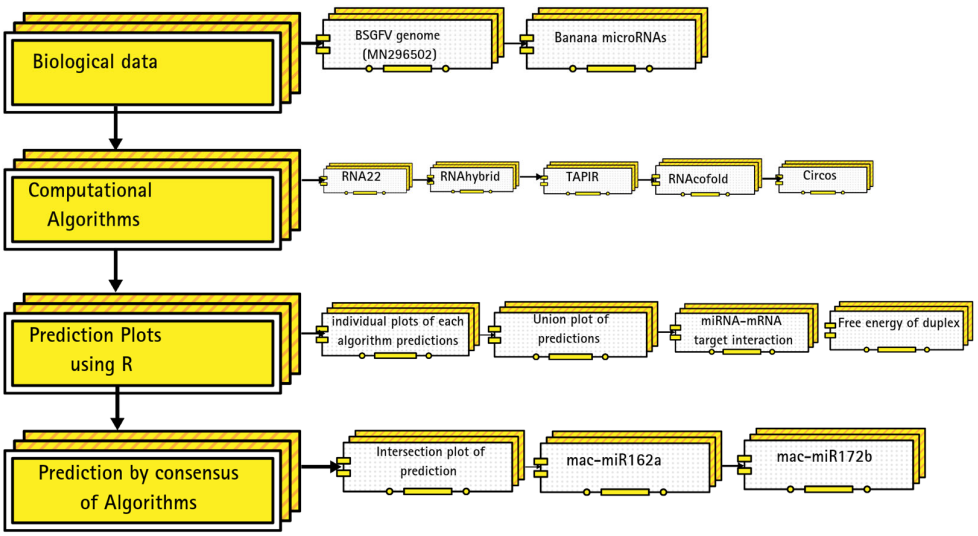

Figure 1. A computational framework designed for predating banana miRNAs that could potentially target BSGFV genome. The biological data consist of banana miRNAs and BSGFV genomic transcript. An algorithmic framework is composed of multiple in silico tools for prediction, interaction and validation of miRNA target binding sites. The R language was used to create plots.

In our previous study, we assessed the sequencing of the BSGFV ORF genome to reveal infection in banana cultivars. We accessed the BSGFV genome from the NCBI Genbank database, and the annotation of protein-coding ORFs was performed. The BSGFV genome carries the largest double-stranded (ds) DNA, plus a strand with a nucleotide content of 7325 bp was drawn that exhibits a significant genetic shift from different isolates. The BSGFV ORF genome consists of three ORFs (Figure 2). Multiple and highly promiscuous banana-encoded miRNA-binding sites were identified in the BSGFV ds DNA genome.

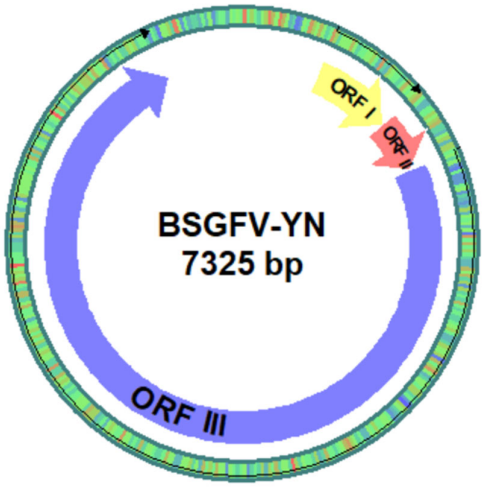

Figure 2. Genome organization of the banana streak GF virus. The whole genome of BSGFV is composed of a ds-DNA molecule of 7325 bp. Schematic diagram of BSGFV ORF genome was generated by pDRAW32 software (v 1.1.152).. The BSGFV encoded three ORFs. The predicted ORFs are denoted with colored arrows. Coordinates are designed based on BSGFV NCBI accession number MN296502. The predicted ORFs of the BSGFV genome indicate the ORFs' distribution throughout genome.

The computational approach to identifying the binding affinity and significance of the 32 banana-encoded miRNAs to the BSGFV dsDNA genome was designed using an integrated in silico predictive approach involving "three algorithms".

In total, 12 banana-encoded miRNAs targeting 22 loci were detected by the RNA22 algorithm. The RNAhybrid algorithm identified 32 banana-genome-encoded locus-derived mac-miRNA/mbg-miRNA–target pairs. Suitable candidate miRNA from banana (mac-miR172b) was observed to target the BSGFV dsDNA genome at a single loci nucleotide position (nt). The TAPIR algorithm predicted potential target site of mac-miR172b at the nt position (7–26) (Figure 3) (Supplementary Table S1 and File S2).

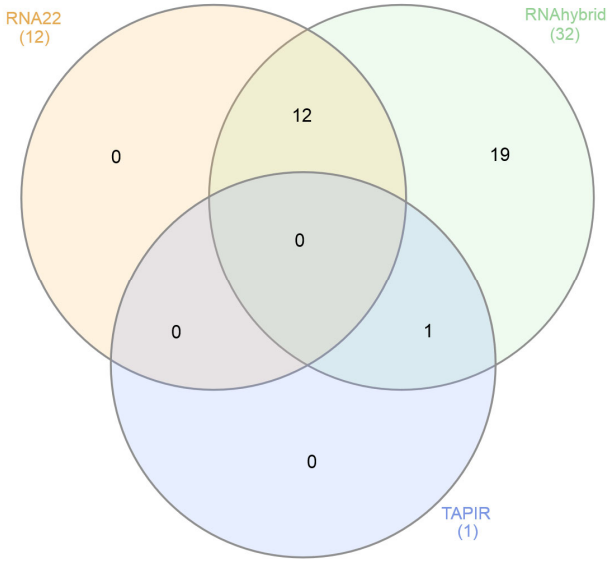

Figure 3. Three-set Venn diagram showing number of mutually common high-confidence binding sites of banana-locus-derived miRNAs predicted to interact with BSGFV-dsDNA-encoded mRNAs. Twelve potentially "efficient" common banana miRNAs were predicted to target multiple sites throughout the viral genome, indicated by RNA22 and RNAhybrid algorithms. No common banana miRNA was identified by all the algorithms used in this study.

3.2. Viral ORF1-Encoding Hypothetical Protein

The badnavirus ORF1 484–1011 (527 nt) encodes the hypothetical protein and includes a domain of unknown function [39]. The ORF1 was targeted by three predicted banana-locus-derived miRNAs: mbg-miR159 (locus 971), mbg-miR399a (locus 603) and mac-miR4995 (locus 656) based on the RNA22 algorithm (Figure 4A). A suitable candidate miRNA from banana was observed to target ORFI at a single loci nucleotide position. The RNAhybrid algorithm identified the binding affinity of mac-miR156g to target ORF1 at nt position 653 (Figure 4B). No banana miRNA was predicted to target the ORF1 gene with the TAPIR tool (Figure 4C and Table 1) (Table S2).

Table 1. The number of banana miRNAs was predicted targeting different ORFs of the BSGFV genome.

BSGFV Genes	RNA22	RNAhybrid	TAPIR
ORF1	mbg-miR159, mbg-miR399a, mac-miR4995	mac-miR156g	
ORFII	mbg-miR159	mac-miR156a-3p, mac-miR156h-3p	

ORFIII	mac-miR156a-3p, mac-miR156h-3p, mac-miR319m, mac-miR160a, mac-miR160g-5p, mac-miR162a, mac-miR164e, mac-miR166b, mac-miR399a1, mac-miR4995	mac-miR156a-5p, mac-miR157b, mac-miR159, mac-miR319m, mac-miR160a, mac-miR, mac-miR	
IR	mac-miR4995		mac-miR172b

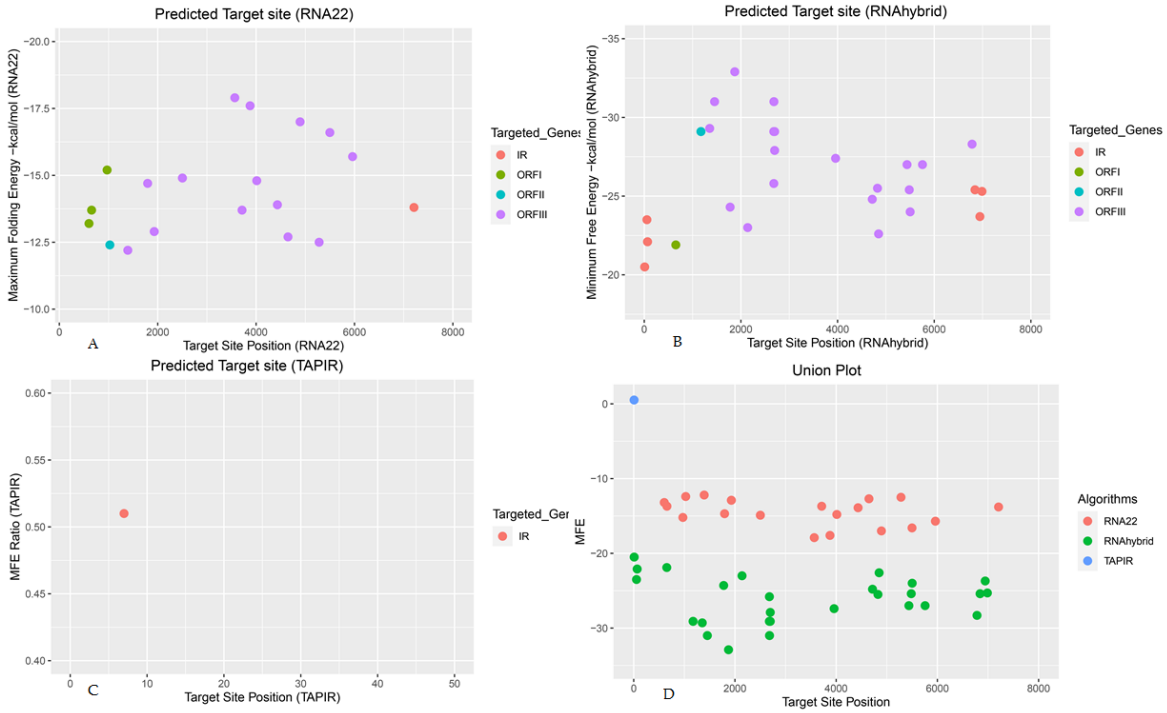

Figure 4. Individual banana-encoded miRNA mac-miRNA/mbg-miRNA and their predicted binding sites were searched in the genomic ds DNA of BSGFV. Three promising miRNA predictions in silico tools were employed, including (**A**) RNA22 algorithm. (**B**) RNAhybrid algorithm. (**C**) TAPIR algorithm to detect high-confidence miRNA-binding sites in the BSGFV genome. (**D**) Union plot showing all predicted banana-encoded miRNA binding sites. Targeted ORFs are indicated by colored dots. Multiple of copies of miRNA-binding sites are shown in colored dots.

3.3. Viral ORF11-Encoding DNA Binding Protein

The badnavirus ORFII (1008–1346 nt) of 338 bases in size encodes a nucleic acid (DNA)—binding protein [39]. Among the BSGFV ORFs targeted, ORFII had very few hybridization binding sites. RNA22 predicted the binding of mbg-miR159 to target ORFII at nt position 1027 (Figure 4A). Similarly, RNAhybrid identified two mac-miRNAs to target the ORFII: mac-miR156a-3p and mac-miR156h-3p at common nucleotide position 1173–1193 (Figure 4B). The miRNA prediction results revealed that no banana-encoded miRNA was detected to target the ORFII region with the TAPIR tool (Figure 4C and Table 1).

3.4. Viral ORFIII-Encoding Polyprotein

The badnavirus ORFIII (1343–6841), consisting of 5498 bases, encodes a polyprotein that is cleaved to functional sub-unit proteins. These include viral capsid and movement protein domains, aspartic protease, reverse transcriptase and RNAse H. The polyprotein functions as a pathogenicity determinant [40].

Eleven miRNAs were predicted for the silencing of the polyprotein (ORFIII) by RNA22: mac-miR156a-3p (locus 2502), mac-miR156h-3p (locus 2502), mbg-miR159 (locus 1027), mac-miR319m (locus 5961), mac-miR160a (locus 1929 and 4433), mac-miR160g-5p

(locus 1929 and 4433), mac-miR162a (locus 3714, 4648 and 5499), mac-miR164e (locus 1795 and 4894), mac-miR166b (locus 5281), mbg-miR399a1 (locus 3567) and mac-miR4995 (locus 1392, 3878 and 4013) (Figure 4A) (Table S2).

Several "potentially efficient" miRNAs in the banana genome were detected to silence the ORFIII by the RNAhybrid algorithm: mac-miR156a-5p (locus 4849), mac-miR157b (locus 1873), mac-miR159 (locus 1354), mac-miR319c (locus 2698), mac-miR160a (locus 2683), mac-miR160a-5g (locus 2683), mac-miR160g (locus 1777), mac-miR162a (locus 5502), mac-miR164e (locus 1455), mac-miR166 (locus 5437), mac-miR166b (locus 6783), mac-miR166c-5p (locus 3959), mac-miR166c-3p (locus 5757), mac-miR167c (locus 2681), mac-miR167d (locus 2680), mac-miR397a (locus 2140), mac-miR399a (locus 5484), mac-miR399a1 (locus 4718) and mac-miR4995 (locus 4826) (Figure 4B). No banana-locus-derived miRNA was detected by the TAPIR (Figure 4C and Table 1)

3.5. Banana miRNAs Targeting Intergenic Region of BSGFV Genome

An intergenic region (IR) of *Badnaviruses*, located between ORF1 and ORV3, has the potential to be used as a promoter. The predicted candidate miRNA from banana (mac-miR4995) was detected that targets the BSGFV IR at nt positions 7209–7230 based on the RNA22 algorithm (Figure 4A).

The RNAhybrid algorithm analysis predicted that the BSGFV ORFIII was targeted by nine miRNAs: mac-miR156, mac-miR156d, mac-miR157b-5p, mac-miR162, mac-miR162b, mac-miR169h, mac-miR172b, mac-miR172c and mac-miR5538 at nucleotides 6945, 68, 68, 6844, 6844, 6988, 9, 9 and 55, respectively (Figure 4B). The TAPIR algorithm identified a hybridization binding site of mac-miR172b at locus position 7 (Figure 4C and Table 1).

3.6. Identification of Unique Banana-Encoded miRNAs

Based on the prediction of high-confidence banana-encoded miRNA binding sites, twelve miRNAs (mac-miR156a-3p, mac-miR156h-3p, mac-miR159, mac-miR319m, mac-miR160a, mac-miR160g-5p, mac-miR162a, mac-miR164e, mac-miR166b, mbg-miR399, mbg-miR399a1 and mac-miR4995) were detected by at least two algorithms considered in this study (Figure 3 and Table 2).

Table 2. Effective hybridization between BSGFV ORFs and their corresponding consensus banana-encoded miRNAs were predicted by all tools.

miRNA ID	Binding Site/ORF RNA22	Binding Site/ORF RNAhybrid	Binding Site/ORF TAPIR	MFE * RNA22	MFE ** RNAhybrid	MFE ** Ratio
mac-miR162a	5499 (ORFIII)	5502 (ORFIII)		−16.6	−24.00	
mac-miR172b		9 (IR)	7(IR)		−20.5	0.51

***** MFE represents maximum folding energy. ** MFE shows minimum free energy.

3.7. Predicting Consensual Banana miRNAs and Silencing BSGFV Genome

Three distinct algorithms RNA22, RNAhybrid and TAPIR were used to identify high-affinity banana-miRNA binding sites in the BSGFV gnome. The consensus is expected to show robustness for predicting conserved antiviral miRNAs in the banana genome. Out of the 32 banana-locus-derived miRNAs investigated, only two conserved banana-genome-encoded miRNAs, mac-miR162a and mac-miR172b, were detected at unique nucleotide positions 5502 and 9, respectively, and as high-confidence hybridization binding sites located in the BSGFV genome consensus based on the combined results of two algorithms, making them the only unique miRNAs identified in this study (Table 2).

The RNAhybrid algorithm was used to estimate the thermodynamically stable hybridization MFE of the consensus miRNA–mRNA pairs: mac-miR162a (−24 Kcal/mole) and mac-miR172b (−20.5 Kcal/mole) and the mode of target inhibition [41] (Table 2).

3.8. Association of Banana miRNA–Target Interaction

A Circos ("Circos" software) map was generated to identify host–miRNA targets. To validate a comprehensive visualization of the host–virus interaction, the predicted Circos map represents biologically credible information that integrated mapped banana miRNAs interacting with BSGFV genomic target genes (ORFs) (Figure 5).

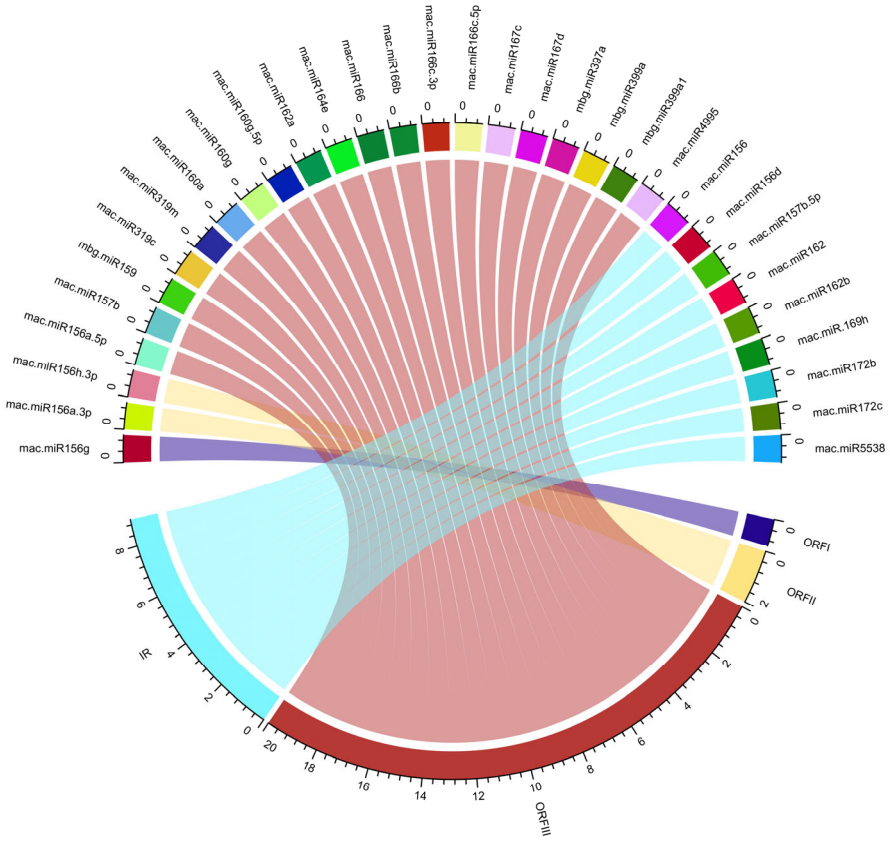

Figure 5. An integrated interaction map shows miRNA–mRNA target interactions. BSGFV ORFs are represented with colored lines. Circos is gold standard software to build circular maps.

3.9. Evaluation of Free Energy (ΔG)

Free hybridization energy (ΔG) of the consensual banana miRNAs and their corresponding viral targets were estimated for a perfectly complementary to validate miRNA–mRNA interactions (Table 3).

Table 3. The free energy (ΔG) of interaction was estimated.

miRNA ID	miRNA–mRNA Sequence (5′–3′)	ΔG Duplex (Kcal/mol)	ΔG Binding (Kcal/mol)
mac-miR162a	5′ GGAUGCAGAGGUUUAUCGACC 3′ 5′ AGATGGACAACTGCTTCCGAG 3′	−20.05	−15.92
mac-miR172b	5′ UGAAUCUUAAUGAUGCUACA 3′ 5′ GAGCAAGGTTAAGATTGATGG 3′	−14.30	−13.11

4. Discussion

The BSGFV is a monopartite *Badnavirus* that spread to China and exhibited a general reduction in productivity and fruit quality. The BSGFV contributed to the epidemic of BSD in China [5,6,42]. A multitude of studies have investigated the expression of potential endogenous host locus-derived mature miRNAs that may target host–plant DNA or RNA viruses based on in silico criteria [43–45]. We observed the computational efficiency of multiple in silico tools used here to identify the binding strength of banana-encoded miRNAs for the screening of a large number of false-positive results. The limitations and bottlenecks of existing in silico tools were evaluated at the individual, intersection and union levels of predictions. This strategy results in the "most effective approach" for analyzing banana-encoded miRNA prediction findings. The miRNA targeting is based on the perfect base-pairing pattern of a miRNA–mRNA seed region [46].

Also, this study examined mature banana-encoded miRNAs that were hybridized with the BSGFV-dsDNA-encoded mRNAs to evaluate promising "seed binding sites" and to elucidate potential interactions with the ORF1, ORFII and ORFII of BSGFV. MicroRNA-based target prediction remains a hotspot in computational biology research. The thermal stability plays the former role in prediction analysis. RNAhybrid is used to calculate "free energy" as the first step for evaluation and includes "seed region" as a key biological element for miRNA–target prediction [34]. We calculated the MFE of consensual miRNA–mRNA target pairs—mac-miR162a (−24.0 Kcal/mol) and mac-miR172b (−20.5 Kcal/mol), with high stringency using the RNAhybrid algorithm (Tables 2 and S1). These results indicate support for the effective interaction of miRNA–mRNA duplexes as the "true target" [47,48]. Further, the "mode of target inhibition" of consensual target pairs was determined as recommended by Broderson [41]. Based on the BSGFV dsDNA genetic sequence, the mature banana-genome-encoded miRNAs mac-miR162a and mac-miR172b are predicted to target BSGFV toward developing streak resistance in banana (Table 2). Our study aimed to identify consensual banana-genome-encoded miRNAs to interact with the ORFIII and IR of the BSGFV genome. We first present a general framework of the association of banana-encoded miRNA targets to capture the complexities of interactions (Figure 5). To model the system-wide virus–host interaction, computational approaches have been employed to identify the binding affinity of host miRNAs for targeted pathogens such as SCMV [49], CLCuKoV [43], RTBV [50], ACLSV [45], ICMV [44], GBNV [51], RTV1 [52], SCYLV [53], ZYMV [54] and PhCMoV [55].

In our previous studies, we undertook the whole-genome sequencing (WGS) of BSGFV [5] in this study; banana-locus-derived potential high-confidence miRNA-binding sites were predicted from the BSGFV genome to validate miRNA–mRNA hybridization (Table 1). Our studies indicate that banana miRNAs are predicted to bind directly with high-confidence sites on BSGFV-dsDNA-encoded mRNAs (Figure 4). Further, in previous studies, we find no evidence of banana-genome-encoded mac-miRNAs that have a predicted potential of targeting BSV genome. Moreover, based on predicted miRNA binding sites, we found that two consensus endogenous miRNAs, mac-miR162a and mac-172b, were identified as highly promising biomolecules andhave an even higher chance to participate in virus replication. The predicted consensual binding site of mac-miR172b is located in the vital regulatory region such as the IR of the BSGFV genome (Figure 4B,C). Notably, the replication of the BSV *Badnavirus* relies on the duplication of ORFIII [11]. mac-miR162a was detected to be effective in interacting with mac-miRNAs targeting the ORFIII for the silencing of the polyprotein of the BSGFV genome (Table 2). mac-miR162a was involved to exhibit the highest overall expression in banana fruit [29]. miR162 regulates stomatal conductance in tomato [56]. The over-expression of osa-miR162 fine-tunes rice immunity against fungal infection [57].

The host-delivered RNAi was employed to develop banana varieties to combat viral infection and offers an extremely effective strategy to enhance banana production [58]. The over-expression profile of miR172 has been reported to reduce red coloration in multiple apple tissues and inhibits flavonoid biosynthesis [59]. miR172 might be involved in carotenoid biosynthesis and involved in regulating biological clock during cold stress in wild banana [60]. miR172b is involved in salt-stress response in rice and wheat crops [61]. Previous study shows that the over-expression of miR172 may lead to the inhibition of *SIAP2a* for a high production of ethylene. This process may cause fruit ripening [62]. The over-expression of miR172 suppresses the brassinosteroid signaling [63]. miR172 is involved to regulate both vegetative and reproductive development in *Jatropha* [64].

While interactions between miRNA–mRNA target pairs have been depicted, the construction of amiRNA-based expression cassettes and further transformations in banana to combat BSGFV infection are not fully understood. The amiRNA expression cassette relies on the potential complementarity of base pairing in order to reduce off-targets toxicity [65,66]. The present study highlights that the strengths and weaknesses of the first genome-based in silico evaluation of mature banana-encoded miRNAs to detect high affinity miRNA-binding sites are based on the energetics for miRNA–target binding. By applying an in silico analysis, experimental work was designed to test whether these predicted consensual miRNAs could impart resistance against BSGFV in banana plants. Furthermore, our earlier research work provides large-sale in silico support for the control of *Badnaviruses* [67,68]. Future work will be focused on understanding the mechanisms of important host–virus-related interactions.

5. Conclusions

BSGFV infects banana plants worldwide. BSGFV appeared as a major problem in China, associated with the globally emerging ongoing infectious endogenous BSV epidemic that is diminishing quantitative yield. This research reports the in silico identification and characterization of banana-genome-encoded mac-miRNAs/mbg-miRNA that is predicted to show efficacy against the BSGFV ORF genome. Prior to cloning, potential banana-locus-derived high-confidence miRNA–target binding sites were identified in the targeted BSGFV ORF genome. Three "algorithms" including RNA22, RNAhybrid and TAPIR were used to detect high-confidence banana-genome-encoded miRNA-binding sites in the BSGFV genome. Among the 32 banana miRNAs investigated, 2 consensus banana-locus-derived mac-miRNAs (mac-miR162a and mac-miR172b) were identified as highly promising, naturally occurring biomolecules with predicted potential to impede BSGFV-YN replication. This study develops an innovative approach to focus on the development of amiRNA-based mac-miRNA therapeutics to target the BSGFV ORF genome. Hence, the comprehensive miRNA structure and interactome mapping of banana miRNA–mRNA target interaction is critical to understand infection and disease progression.

Supplementary Materials: The following supporting information can be downloaded at www.mdpi.com/10.3390/applmicrobiol5010013/s1. Table S1: Identification of binding sites of mature banana-encoded miRNAs; Table S2: Gene-wise of predicted miRNAs; File S1: Banana mature microRNA sequences; File S2: Prediction results analyzed by computational algorithms.

Author Contributions: M.A.A. and N.Y. conceived of the concept, approaches and data interpretation. M.A.A., A.S., E.S. and S.I. carried out the in silico experiments. B.A., S.B. and M.F. made the graphs. M.A.A. made the tables and wrote the manuscript. All of the authors participated in the interpretation of the results. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the International Science and Technology Cooperation Program of Hainan Province (GHYF2023010), Hainan Provincial Natural Science Foundation of China (322RC769) and Central Public Interest Scientific Institution Basal Research Fund (No. 1630052023003).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials.

Acknowledgments: The authors acknowledge the TYSP of China to support M.A.A.

Conflicts of Interest: The authors declare they have no conflicts of interest.

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