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Candidate miRNAs from *Oryza sativa* for Silencing the Rice Tungro Viruses

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Abstract: Rice tungro disease (RTD), caused by *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV) is one of the most prominent viral diseases in Asian countries. This virus disease problem seems to have been accentuated in those countries by causing a series of outbreaks over the years after being first reported in International Rice Research Institute (IRRI), Philippines, in 1963. One of the effective ways to combat viruses is through RNA silencing. microRNA is an important player in the RNA silencing mechanism. Genome sequences analysis shows RTBV-SP isolate (8 Kb) is composed of four open reading frames (ORF 1, ORF 2, ORF 3, and ORF 4), meanwhile, RTSV-SP (12 Kb) consists of one open reading frame encoded by seven different polyproteins (P1, CP1, CP2, CP3, NTP, Pro, and Rep). Therefore, this study investigated possible rice-encoded miRNAs targeted on RTBV and RTSV using in silico analysis. Five bioinformatics tools were employed using five different prediction algorithms: miRanda, RNA22, RNAhybrid, Tapirhybrid, and psRNATarget. The results revealed each RTBV and RTSV can be silenced by three potentially best candidate rice-encoded miRNA. For RTBV, osa-miR5510 (accession no. MIMAT0022143), osa-miR3980a-3p (accession no. MIMAT0019676), and osa-miR3980b-3p (accession no. MIMAT0019678) are being predicted by all five algorithms. Meanwhile, for RTSV, three miRNAs predicted are osa-miR414 (accession no. MIMAT0001330), osa-miR5505 (accession no. MIMAT00221138) and osa-miR167a-3p (accession no. MIMAT0006780). The predicted data provide useful material for developing RTBV and RTSV-resistant rice varieties.

Keywords: rice tungro disease; bioinformatics; miRNA; *Oryza sativa*

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1. Introduction

Rice (*Oryza sativa* L.) is a cereal crop from the family Poaceae and the staple diet to over half the global population. Its consumption in Asian countries is expected to increase up to 650 million tons by 2050; thus, more rice production is needed along with the rapid growth of the population [1]. Being a staple crop grown worldwide, rice is exposed to a wide range of pathogens, thus leading to yield loss and high production costs. Rice pathogens include bacteria, fungi, and viruses that could cause catastrophic disease in rice communities. Viruses are among the most prominent agents of infectious diseases in rice. Various viral disease symptoms can affect rice, including stunting, yellowing of leaves, and other developmental abnormalities such as ringspot, leaf rolling, wilting, and necrosis [2]. Multiple viruses from different families that cause substantial yield loss worldwide are being detected and thoroughly studied to improve damage management in the rice industry [3].

Among other virus-related diseases, Rice tungro disease (RTD) is an economically significant viral disease in Southeast Asian rice-growing countries. This virus disease

problem seems to have been accentuated in those countries by causing a series of outbreaks over the years after being first reported in International Rice Research Institute (IRRI), the Philippines, in 1963 [4]. Later, the disease caused outbreaks recorded in several rice production countries such as India, Indonesia, Malaysia, Philippines, China, Thailand, and Bangladesh [5,6]. The first outbreak of rice tungro diseases, or "Penyakit Merah" in Malaysia was reported in Kerian in 1933. A major outbreak was reported in 1982, affecting more than 20,000 ha of Kedah and Perlis rice fields [7]. According to Yee and Eng [8], a recent RTD invasion in Sarawak was discovered in 2012.

RTD is transmitted by a synergistic effect of two distinct viruses; *Rice tungro bacilliform virus* (RTBV) and *Rice tungro spherical virus* (RTSV). RTBV is a type of pararetrovirus classified into the *Tungrovirus* genus and belongs to the family of *Caulimoviridae* consists of a double-stranded DNA genome. Meanwhile, RTSV, a picornavirus from the genus *Waikavirus* and family *Secoviridae* has a single-stranded RNA genome [9–13]. RTBV and RTSV are transmitted by an insect vector, green leafhopper (*Nephotettix virescens*), GLH. GLH would transmit the viruses as a complex or separately, whereas RTSV can be transmitted independently; meanwhile, RTBV requires RTSV or its helper factors for the disease transmission. The RTBV is principally responsible for the symptom's development, causing changes in plant growth, while the RTSV encodes proteins necessary for the virus transmission between individual plants. An RTBV-RTSV interaction induces disease symptoms, including stunting and discoloration of diseased plants, decreased tillering and sterile panicles [14,15].

RTBV has an 8000 bp double-stranded DNA genome with four open-reading frames (ORFs) [16]. To date, the whole sequence of RTBV strain was first sequenced in 1991 (Phi-1), originated from IRRI, then followed by five different biological variants of RTBV, Phi-2 [17], Phi-3 [18], G1, Ic, and G2 [14]. Meanwhile, several isolates originated from India were then reported, such as Chinsura [19], Kanyakumari [20], Punjab [21], Andhra Pradesh, and West Bengal [22]. In addition, complete genomes of isolates from Chainat, Serdang [23], and Seberang Perai [24] have also been reported. RTSV has a 12,000 bp single-stranded RNA consisting of only one ORF coded for seven different proteins.

Plants have evolved complex defence mechanisms to protect themselves from viral attacks. One of the effective ways to combat viruses is through RNA silencing. RNA silencing refers to a mechanism of the eukaryotic genome mainly mediated by microRNAs (miRNAs), which causes down-regulation of gene expression [25]. miRNAs consist of 20- to 24- nucleotide small RNAs, genome-encoded molecules that regulate gene regulation at the post-transcriptional level in eukaryotes [26,27]. Through a plant's innate immune system, its mechanism treats the virus as a gene that is being expressed out of control. This silencing pathway involves either suppressing gene transcription or degrading RNA species in response to abiotic stress [28]. Moreover, the mechanism requires a series of protein components such as Dicer-like (DCL) RNase-III endonucleases, Argonautes proteins (AGO), RNA-dependent RNA polymerase (RdRP) and RNA-induced silencing complexes (RISC) [3].

Viral infection causes an alteration in the miRNA level of expression in plants [29]. Thus, these miRNAs that undergo changes in their expression may play potentially essential roles in defending the plant system from viral invasion and replication. Due to the importance of miRNAs in reaction to any stressful condition, including viral invasion, more than 6000 miRNAs in approximately 60 plant species have been identified [27]. Furthermore, the current pipeline in the identification of miRNA blends interdisciplinary paths involving biological and bioinformatics approaches.

The viruses were targeted by abundant and conserved miRNA families in regions coding for various functioning proteins, it is suggested that computational approaches play an important role not only in the discoveries of miRNA genes but also in the identification of miRNA targets. Computational approaches are currently being developed to find the regulatory targets of the miRNAs, providing clues to miRNA function based on the known roles of these targets. This method has been applied in predicting miRNA to combat

plant viruses such as *Physostegia chlorotic mottle virus* (PhCMoV) and *Tomato brown rugose fruit virus* (ToBRFV) [30], *Maize chlorotic mottle virus* (MCMV) [31], *Sugarcane bacilliform virus* (SCBV) [32], and cassava mosaic disease (CMD) [33]. Tripathy et al. were able to predict the rice miRNA target sites against tungro viruses by using various string-matching algorithms [34]. The same approach also has been conducted in other rice viruses such as *Rice yellow mottle virus* (RYMV) [35]. Therefore, for providing additional discovery in viral disease of rice, this research aims to implement computational or in silico methods for identifying the target rice miRNAs in the genome of RTBV and RTSV. This result can provide the best rice miRNA candidate for enhancing rice plants' resistance to tungro disease through RNA-mediated techniques in producing disease resistant plants such as RNA-interference, the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas), and so on.

2. Materials and Methods

2.1. Genome Sequences and Mature miRNAs Retrieving

The available complete genome sequences of *Rice tungro bacilliform virus* (RTBV) Seberang Perai, Malaysia isolate (RTBV; accession no. MK552377.1) [24] and *Rice tungro spherical virus* (RTSV) Seberang Perai, Malaysia isolate (RTSV; accession no. MK655459.1) [36] were accessed from NCBI GenBank. A total of 747 rice (*O. sativa*) mature miRNA and their sequences were acquired from the miRBase database (https://www.mirbase.org/, accessed on 22 June 2022).

2.2. Sequence Analysis of RTBV and RTSV Genome

The retrieved RTBV and RTSV genome sequences were aligned using the MUSCLE multiple aligners available in the Galaxy Europe server (https://usegalaxy.eu/, accessed on 22 June 2022) [15,37]. The maximum number of iterations to be run (-maxiters) by MUSCLE was set to 16. The nucleotide distribution in the genomes was annotated and visualized on QIAGEN CLC Genomics Workbench 20. ORF of each viruses' genome was determined using the ORFfinder tool (https://www.ncbi.nlm.nih.gov/orffinder/, accessed on 26 June 2022).

2.3. MicroRNA Target Prediction in RTBV and RTSV Genome

Five web-based bioinformatic tools were used in this analysis. Those five different tools are miRanda, RNAhybrid, RNA22, Tapirhybrid, and psRNATarget. The parameters and filtering used for each tool are shown in Table 1. In addition, the precursors and secondary structure of the nominated miRNAs' sequences were predicted using RNAfold [38].

Table 1. Parameters used for each rice microRNA target site prediction algorithm in this study.

Tool	Parameter	Source	Reference
miRanda	Alignment score threshold = 140, energy threshold = −20 kcal/mol.	https://usegalaxy.eu/ (accessed on 24 June 2022)	[37]
RNAhybrid	The energy threshold = −20 kcal/mol.	https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/ (accessed on 23 June 2022)	[39]
RNA22	The minimum number of paired-up bases = 12, maximum folding energy = −14 kcal/mol.	https://cm.jefferson.edu/rna22/Interactive/ (accessed on 24 June 2022)	[40]
Tapirhybrid	Score = 8, free energy ratio = 0.5.	http://bioinformatics.psb.ugent.be/webtools/tapir/ (accessed on 23 June 2022)	[41]
psRNATarget	Expectation score = 7.0, penalty for other mismatches = 1, penalty for G.U pair = 1, seed region = 2–7 nucleotides, HSP size = 19, penalty for extending gap = 2.	http://plantgrn.noble.org/psRNATarget/analysis?function = 3 (accessed on 25 June 2022)	[42]

2.4. Statistical Analysis

R statistical software was used to analyse rice miRNA predicted data acquired from all five bioinformatics methods [43]. The ggplot2 software was used to provide a graphical representation of the results [44].

3. Results

3.1. Analysis of RTBV Genome

The full-length genome of the Seberang Perai isolate (accession no. MK552377.1) was sequenced for the first time by Kannan et al. after being obtained from infected fields in Seberang Perai, Malaysia [24]. It consists of 8000 nucleotides in full length. With a G + C content of 33.3%, its genome sequence of RTBV-SP has the highest nucleotide similarity with the Serdang isolate (accession no. AF076470). Based on the visualization of the genome by CLC Genomics Workbench 20, RTBV contains four open reading frames (ORFs) (Figure 1), each of which is capable of encoding proteins with a molecular mass of 24, 12, 194, and 46 kDa [16]. Arcs show the positions of ORFs within the double-stranded DNA genome. ORF 1 is coded at 68–667, ORF 2 is coded at 652–996, ORF 3 is coded at 978–6014, and ORF 4 is coded at 5948–7204. On the polyprotein ORF 3 (P194), movement protein (MP), chromosome segregation protein (PRK03918), aspartate protease (PR), reverse transcriptase (RT), and RNase H (RH) are located. In addition, the P12 protein is located on ORF 2, meanwhile, the P46 protein is located on ORF 4.

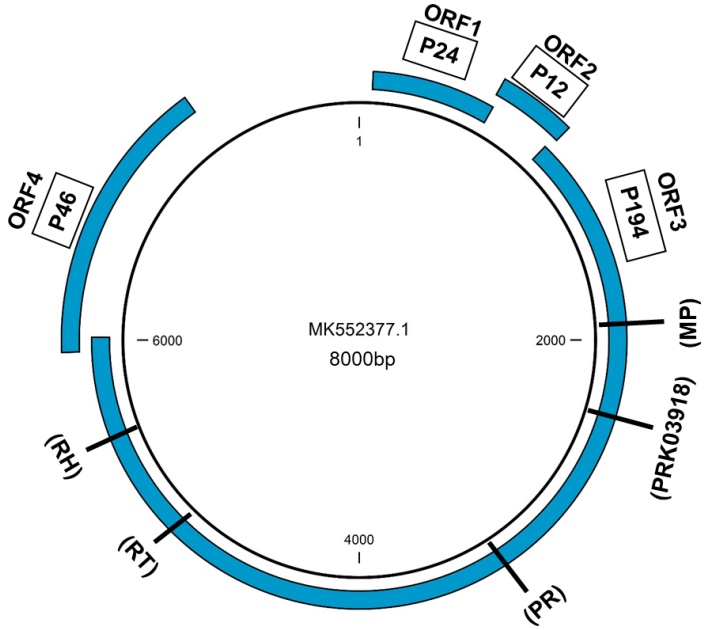

Figure 1. Genomic organization of RTBV-SP (RTBV: accession no. MK552377.1). The open reading frames are shown in blue (ORF 1, ORF 2, ORF 3, and ORF 4).

3.2. Analysis of RTSV Genome

RTSV-SP genome has 12,173 nucleotides in length (accession no. MK655459.1) with a G + C content of 45.82 percent. The nucleotide sequence of the RTSV-SP isolate was found to be 86.22–96.02% identical with other RTSV genomes available in the Genebank database, according to searches through blastn. The highest percentage of identity to RTSV-SP comes from RTSV isolate Vt6 (accession no. AB064963.1). RTSV-SP has only one open reading frame, consisting of different domains. The leader protein (P1) is coded at 515–2437, coat protein 1 (CP1) is coded at 2438–3061, coat protein 2 (CP2) is coded at 3062–3670, coat protein 3 (CP3) is coded at 3671–4546, nucleotide triphosphate binding protein (NTP) is coded at 9062–10,927 (Figure 2).

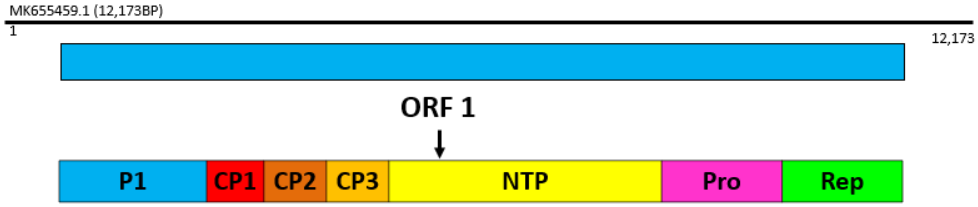

Figure 2. RTSV genome splicing view shows the ORF and the domains.

3.3. MicroRNA Target Prediction in RTBV and RTSV Genome

3.3.1. Host-Derived miRNas Predicted by Different Bioinformatic Algorithms

All algorithms generated miRNA-target pair, MFE (Minimum Free Energy), and locus of targeted position through different work mechanisms (Table 1). Out of 747 mature rice-derived miRNAs, the miRanda algorithm predicted 73 miRNAs targeting 81 loci on the RTBV genome (Figure 3A), RNAhybrid predicted 699 miRNAs targeting 699 loci (Figure 3B), RNA22 predicted 171 miRNAs targeting 220 loci (Figure 3C), Tapirhybrid predicted 54 miRNAs targeting 54 loci (Figure 3D), and psRNATarget predicted 338 miRNAs targeting 611 loci (Figure 3E). Thus, 128 miRNAs have targeted a common region on the RTBV genome predicted by at least three tools. Meanwhile, 45 and 3 rice miRNAs were supported by at least four and five algorithms, respectively. Among all the predicted miRNAs, three miRNAs, which are osa-miR5510 (accession no. MIMAT0022143), osa-miR3980a-3p (accession no. MIMAT0019676), and osa-miR3980b-3p (accession no. MIMAT0019678), identified targeting at the same loci by at least three algorithms used (Tables 2 and 3).

Table 2. MiRanda's algorithm analysed the outcome of miRNA-target pairs. Algorithm generated an miRNA-target pair, MFE (Minimum Free Energy), locus of the targeted position.

Host miRNAs	miRNA-Target Pair	Position	MFE (kcal/mol)
	RTBV-SP		
osa-miR5510	Query: 3' aggagacCUCACCUAGUCGGa 5' Ref: 5' taaaaatGAGTGGATTAGCCt 3'	6262–6282	−25.83
osa-miR3980a-3p	Query: 3' ucuUAGCUGCCGGAGCCGGUc 5' Ref: 5' aacATGCATGGGCTTGGCCAg 3'	7110–7130	−27.26
osa-miR3980b-3p	Query: 3' ucuUAGCUGCCGGAGCCGGUc 5' Ref: 5' aacATGCATGGGCTTGGCCAg 3'	7110–7130	−27.26
	RTSV-SP		
osa-miR414	Query: 3' ccugCUACUACUACUCCUACu 5' Ref: 5' tctgGACGATACTGGGGATGt 3'	1801–1821	−23.16
osa-miR5505	Query: 3' auCGCUAGUUAUGGCUUAGGAg 5' Ref: 5' gaGCAAGCAAAACTGAATGCTc 3'	2084–2105	−21.17
osa-miR167a-3p	Query: 3' uuUACUCCGACAGUACGUACUa 5' Ref: 5' acATGAGGCTGCCATCCATGGa 3'	10,624–10,645	−25.40

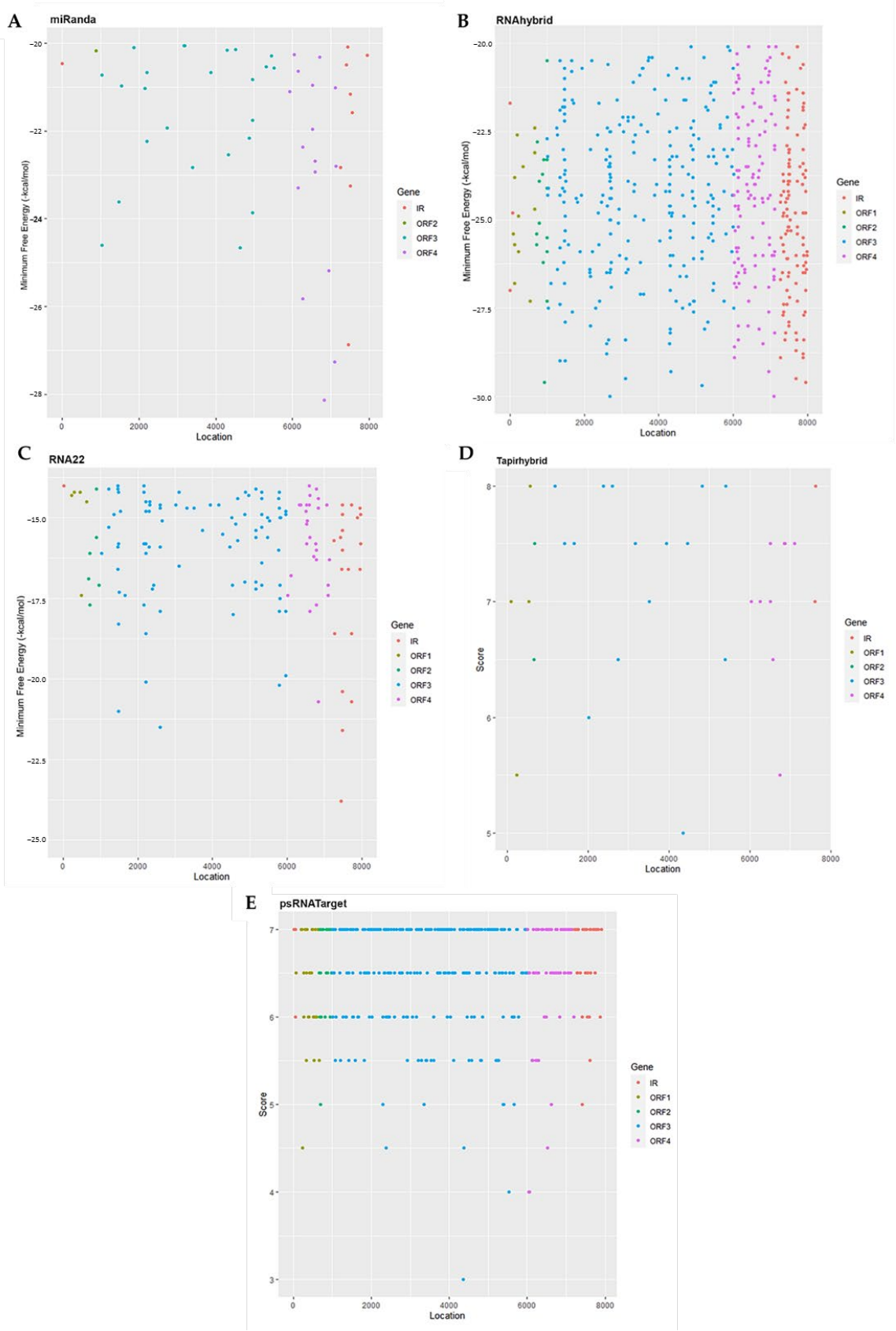

Figure 3. miRNA target site prediction outcome of rice's miRNA on the RTBV's genome sequence using the algorithms of miRanda (**A**), RNAhybrid (**B**), RNA22 (**C**), Tapirhybrid (**D**), psRNATarget (**E**). The colour key represents the respective regions in the genome. IR indicate intergenic region.

Table 3. The best host-derived miRNAs target the RTBV and RTSV genomes and their mature sequence.

Host miRNAs	Accession No.	Mature Sequence
RTBV-SP		
osa-miR5510	MIMAT0022143	AGGCUGAUCCACUCCAGAGGA
osa-miR3980a-3p	MIMAT0019676	CUGGCCGAGGCCGUCGAUUCU
osa-miR3980b-3p	MIMAT0019678	CUGGCCGAGGCCGUCGAUUCU
RTSV-SP		
osa-miR414	MIMAT0001330	UCAUCCUCAUCAUCAUCGUCC
osa-miR5505	MIMAT0022138	GAGGAUUCGGUAUUGAUCGCUA
osa-miR167a-3p	MIMAT0006780	AUCAUGCAUGACAGCCUCAUUU

The miRanda algorithm predicted 85 miRNAs targeting 99 loci on the RTSV genome (Figure 4A), RNAhybrid predicted 720 miRNAs targeting 720 loci (Figure 4B), RNA22 predicted 205 miRNAs targeting 251 loci (Figure 4C), Tapirhybrid predicted 51 miRNAs targeting 51 loci (Figure 4D), and psRNATarget predicted 431 miRNAs targeting 706 loci (Figure 4E). Among all the predicted miRNAs, three miRNAs, which are osa-miR414 (accession no. MIMAT0001330), osa-miR5505 (accession no. MIMAT0022138), and osa-miR167a-3p (accession no. MIMAT0006780), were identified targeting at the same loci by all five algorithms used (Tables 2 and 3). All algorithms also determined the target start position on the genome of RTBV and RTSV by rice-encoded miRNAs (Table 4).

Table 4. The best host-derived miRNAs target the RTBV and RTSV genome, and their target position identified by all algorithms.

Host miRNAs	Start Position				
	miRanda	RNAhybrid	RNA22	Tapirhybrid	psRNATarget
RTBV-SP					
osa-miR5510	6262	6268	5157	6262	6262
osa-miR3980a-3p	7110	7107	7486	7110	7110
osa-miR3980b-3p	7110	7107	7486	7110	7110
RTSV-SP					
osa-miR414	1801	1801	1801	1803	1786
osa-miR5505	2084	2084	2084	2084	2079
osa-miR167a-3p	10,624	10,624	10,624	10,624	10,625

3.3.2. Prediction of Secondary Structure

The secondary structure of the predicted rice miRNAs was obtained using the RNAfold algorithm. Figures show the predicted folding structure of the precursors osa-miR5510, osa-miR3980a-3p, and osa-miR3980b-3p for RTBV (Figure 5) and osa-miR414, osa-miR5505, and osa-miR167a-3p for RTSV (Figure 6).

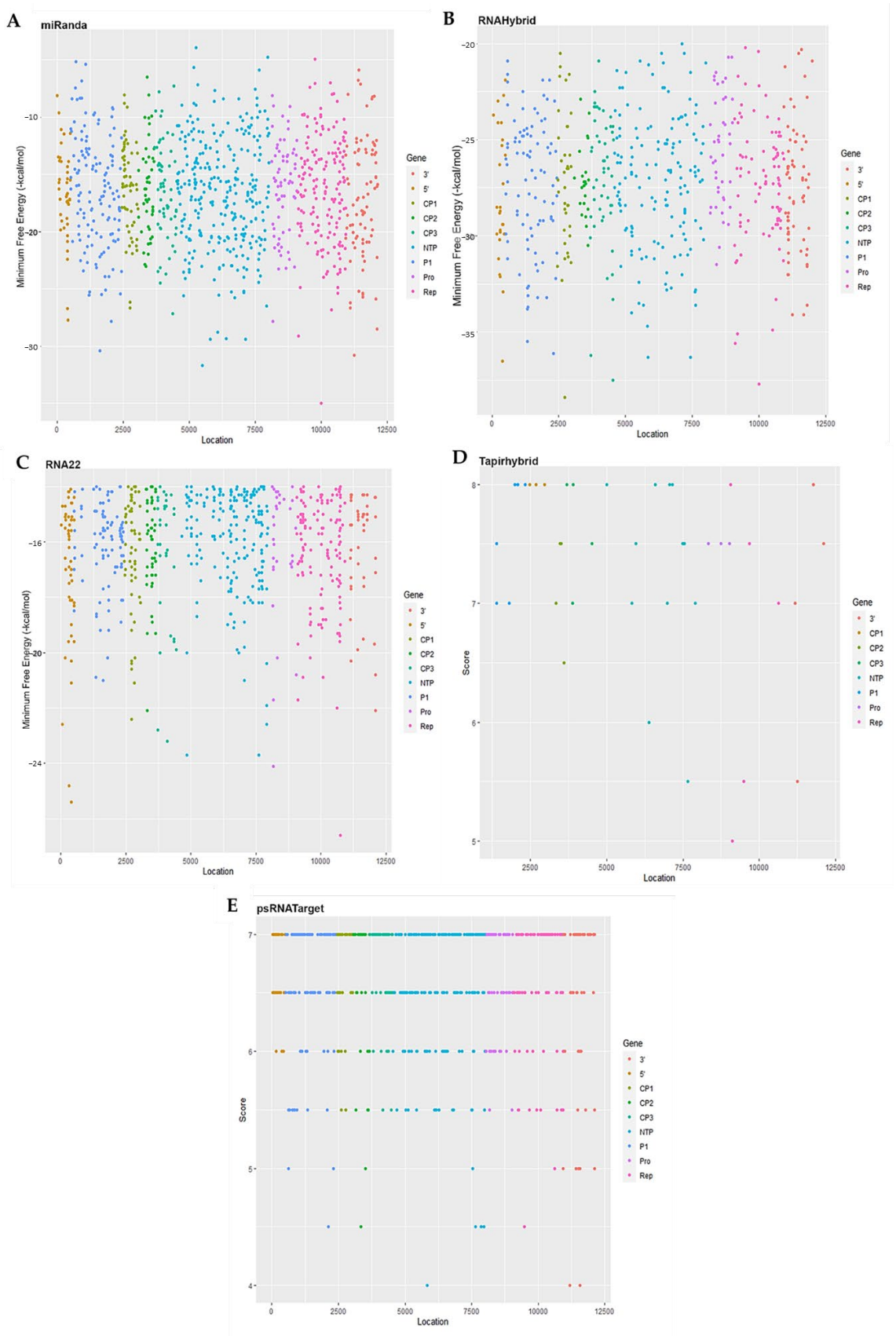

Figure 4. miRNA target site prediction outcome of rice's miRNA on the RTSV's genome sequence using the algorithms of miRanda (**A**), RNAhybrid (**B**), RNA22 (**C**), Tapirhybrid (**D**), psRNATarget (**E**). The colour key represents the respective regions in the genome.

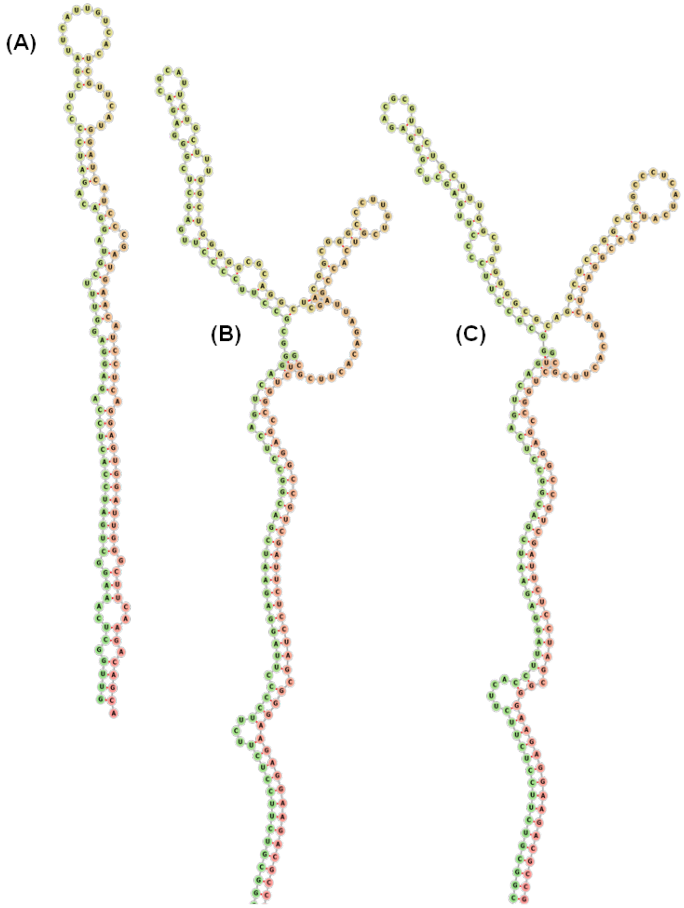

Figure 5. The RNAfold web server predicted the folding structure of the precursor osa-miR5510 (**A**), osa-miR3980a-3p (**B**), and osa-miR3980b-3p (**C**).

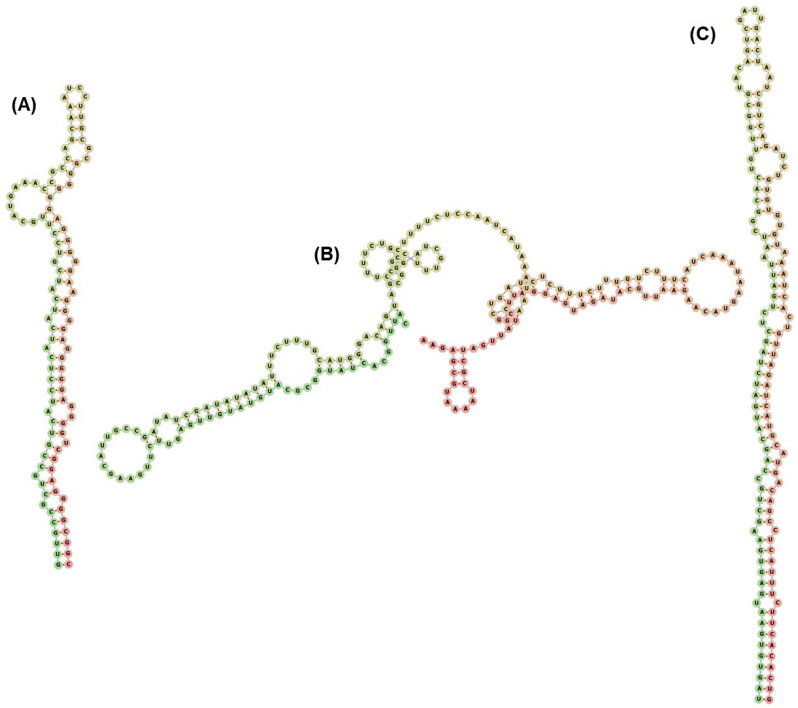

Figure 6. The RNAfold web server predicted the folding structure of the precursor osa-miR414 (**A**), osa-miR5505 (**B**), and osa-miR167a-3p (**C**).

4. Discussion

Similar research employing several computational approaches to determine the optimum miRNA for the targeted pathogen has previously been published to understand plant-virus interaction [30,35]. However, there is limited study focusing on rice plant diseases, specifically rice tungro disease. To provide a way of solution to manage rice tungro disease, this research used the same unique computational technique to anticipate rice-encoded miRNA targets in the RTBV and RTSV genomes. To reduce false-positive results, various techniques were utilised to anticipate probable and more accurate miRNA targets for RTBV and RTSV silencing. These five algorithms are based on minimum free energy parameters that surpass threshold standards, folding energy, accessibility of target site, seed pairing, and pattern recognition and are executed differently. The sequence complementarity assessment, the free energy of the RNA-RNA complex, and cross-species sequence conservation of the miRNA prediction's molecular target site are all taken into account by miRanda. Furthermore, it can predict numerous target loci across a genome [45]. The locus site complementarity, target-site abundance, and minimal free energy are all analysed using RNAhybrid [46].

Meanwhile, RNA22 detects possible miRNA target sites using a different approach than previous tools, a pattern recognition approach, and folding energy to discover the target loci, with the least free energy being the important parameter measured. In contrast to miRanda, RNA22′s final prediction does not rely on cross-species conservation [31,40]. Because of its precise system and quick execution, Tapirhybrid is a well-known and frequently recommended plant miRNA target prediction tool [39,41]. It was designed with numerous important factors, such as seed pairing, accessibility of the target site, and multiple target sites [41]. Based on a scoring system, psRNATarget was created to analyse complementarity between the miRNA sequence and the target mRNA sequence. It then calculates the unpaired energy required to assess target site accessibility [42]. Within the genomes of the two viruses, the five algorithms revealed potential target locations for rice-encoded miRNAs. According to at least one computational tool, all rice miRNAs used in this study have the probability of targeting the genomes of RTBV and RTSV.

osa-miR5510, osa-miR3980a-3p, and osa-miR3980b-3p can target the genome of RTBV at P146 region. osa-miR3980a-3p and osa-miR3980b-3p are nearly identical orthologs, belong to the same family but being expressed by different precursors osa-miR3980a-3p and osa-miR3980b-3p, respectively, as being visualized in Figure 5. Three miRNAs were identified targeting the RTSV genome at the same loci by all five algorithms used. For the RTSV analysis, the osa-miR414 and osa-miR5505 target the P1 region (515–2437), while the osa-miR167a-3p targets the Rep region (9062–10,927). Previous studies show little information about the involvement of osa-miR5510 in regulating vital functions in rice plant development and growth. However, a study has predicted that osa-miR5510 is regulated in developing pollen and sporophytes in rice [47]. osa-miR5510 is also expected to target the ORF 2 region of the *Rice yellow mottle virus* (RYMV) genome in rice to silence the viral polyprotein [35]. It is anticipated that both miR3980a-3p and miR3980b-3p target the pectinesterase gene, and it involves starch and sucrose metabolism affecting pollen fertility and grain filling under heat stress in rice [48]. Furthermore, they were observed to exhibit significant differential expression between the sterile male line and its maintainer line in a study investigating miRNAs' regulatory roles in rice female gametophyte abortion [49]. Both miRNAs have also been differently expressed in rice plants under abiotic stress, such as drought and brown planthopper attack [50,51].

The observation in a previous study suggested that osa-miR414 is targeting transcriptional regulators [52]. The bZIP family transcription factors, WRKY, MBY, B3, heat shock proteins, and TCP are all target transcription factors involved in plant growth and development, morphological and anatomical changes, metabolic processes, and defence responses. The osa-miR414 is also involved in post-transcriptional modifications, such as SNF2 transcriptional regulator, pentatricopeptide repeat-containing proteins, and high mobility groups proteins, C_2H_2 zinc finger proteins, and RNA recognition motifs. A study

shows that osa-miR5505 was found in Vandana and Nagina 22 cultivars rather than in wild rice species, suggesting that this miRNA involves selecting the domestication in cultivated rice [53]. The osa-miR167a-3p revealed a significant link with the grain developmental process through the auxin-miR167-ARF8-OsGH3.2 regulatory pathway [54]. The researchers also discovered that when osa-miR167 was overexpressed, the plant's height, tiller number per individual plant, panicle length, spikelet number per panicle, and seed setting rate were all reduced through controlling the expression of ARF family transcriptional factors. osa-miR167 was also up-regulated during in rice response to sheath blight disease [55]. The up-regulation pattern of osa-miR167 could also be observed during salt, drought, and powdery mildew disease stress in Arabidopsis and wheat, respectively [56,57].

Other candidate miRNAs predicted by at least three algorithms were found to be involved in pathogen infection reactions. In RTBV- and RTSV-infected plants, osa-miR1439 is being down-regulated, and the concurring increases in the expression of its target gene could contribute to innate rice immunity against those viruses [58]. Overexpressed osa-miR167h-3p in rice plants positively regulates the resistance to bacterial blight disease transmitted by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) [59]. It is also up-regulated in the *Rice stripe virus* (RSV), targeting the chloroplast-related gene to defend against the viral infection [60].

Meanwhile, miR398a, osa-miR1320-5p, and osa-miR1432-3p are differentially expressed in response to *Xoo* infection that causes bacterial blight disease in susceptible rice variety and may regulate defence response against the infection [59]. Yang et al. also demonstrated that the osa-miR1870-5p-mediated pathway is being altered by RSV infection by up-regulating the miRNAs and suppressing the defense response in susceptible plants [60]. osa-miR395y and osa-miR395a targeting a disease resistance gene, the NBS-LRR gene that is up-regulated by RSV infection 3–5 days after inoculation [61]. osa-miR2118m from the miR2118 family regulates the NBS-LRR protein-related fungal and bacterial disease resistance [62]. Another member of the miR2118 family, miR2118l was differentially expressed in resistant and susceptible lines in *Magnaporthe oryzae* resistance response in rice. Target of this transcript, NBSGO protein coding gene LOC_Os11g44580 was up-regulated in a resistant line against *M. oryzae*. The same study also reported another candidate, miR395l as one of the negative regulator specific to the resistant line [63]. osa-miR5515 has been detected to target ORF 1 of the *Rice yellow mottle virus* (RYMV) genome, which encodes protein P1 needed for systemic infection and viral replication in rice. At the same time, osa-miR2864.1 has targeted ORF 2 of the same viral genome in silencing the RYMV protein [35].

In *Rhizoctonia solani*-treated rice plants, expression analysis showed osa-miR398a was significantly induced and led to the fungal infection [64]. Osa-miR43h has been reported to be down-regulated with brown planthopper (BPH) infestation, where it is predicted to target α/ß hydrolase associated with the gibberellin pathway in rice defence against the insect. BPH is a pest-causing hopper burn disease that kills the plant by direct feeding [65]. osa-miR5827 predicted by RNAhybrid and psRNATarget algorithm contributed to a low rate of pre-harvest sprouting in rice by targeting the Os03g0728900 (OsbHLH084) genes in the seed embryo. This mechanism would prevent yield loss and low grain quality in rice production countries [66]. Previous studies also implemented the computational approach in predicting miRNA targets for RTBV and RTSV. For example, Tripathy and Mishra have predicted over ten miRNAs using their proposed algorithms, including osa-miR2929 and osa-miR5525, targeting both RTBV and RTSV [34,67].

The predicted rice-encoded miRNAs can lead to the generation of tungro-resistant *O. sativa* by applying transformation strategies. In addition, these miRNAs may also be used to produce functional artificial miRNAs that could improve rice plants' turgidity in the fight against tungro disease. Therefore, future work would validate and clarify these miRNAs by experimental method to obtain complete legitimacy. This will help generate future varieties of *O. sativa* resistant to RTBV and RTSV.

5. Conclusions

A comprehensive and varied computational strategy is employed in this study to identify rice-encoded miRNAs for silencing RTBV and RTSV. Putative miRNAs targeting the RTBV genome have been predicted on the virus genomes using five different bioinformatics programs built with a different algorithm. Among the 747 rice miRNAs available in miRBase, six rice-encoded miRNAs can be candidates for silencing the RTBV or RTSV. This computational approach also helps to find the regulatory target of these miRNAs and provides the function of them based on the known roles of the targets. Furthermore, computationally predicted miRNA and its target is suggested to be supported by subsequent experiments for further validation to complete the study and reduce false positive predictions, if any. This study's findings may contribute to developing rice plants resistant to RTBV and RTSV through all miRNA-utilizing tools and methods.

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