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Communication Article

Naturally Infecting Hibiscus Spp. In Hawaii: The First Identification And Genome Characterization Of A New Dna Virus, Hibiscus Soymovirus, And A New Rna Virus, Hibiscus Betacarmovirus.

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Abstract

In 2020, hibiscus leaves (Hibiscus spp., family Malvaceae) with mosaic, ringspot, and chlorotic spot symptoms were gathered from Oahu, Hawaii. Ribosomal RNA-depleted composite RNA samples taken from symptomatic leaves were subjected to high-throughput sequencing analysis. Following quality checking, trimming, and de novo assembly, over 77 million paired-end reads and 161,970 contigs were produced. Using BLASTX/BLASTN searches for contig annotation, two sequences were found: contig 1 resembled the RNA virus, hibiscus chlorotic ringspot virus (genus Betacarmovirus), and contig 2 resembled the DNA virus, peanut chlorotic streak virus (genus Soymovirus). With the suggested names of hibiscus betacarmovirus and hibiscus soymovirus, respectively, these viruses are thought to represent new species in the genera betacarmovirus and soymovirus, according to additional bioinformatic analyses of the full viral genome sequences.The presence of these viruses was further verified by Sanger sequencing in conjunction with RT-PCR employing particular primers created based on the recovered contigs. To ascertain the prevalence and dispersion of these viruses, 54 more hibiscus leaf samples from various Oahu locales were analyzed.

Keywords : *hibiscus; high-throughput sequencing; betacarmovirus; soymovirus.*

INTRODUCTION

Hawaiian landscapes often feature hibiscus (Hibiscus spp., family Malvaceae, order Malvales) as an ornamental shrub. Several plant viruses, including those belonging to the genera Tobamovirus, Betacarmovirus, Begomovirus, Cilevirus, Higrevirus, and Ilarvirus, have been known to infect this plant. Mosaic, ringspot, and chlorotic patches are signs of infection in plants [1]. There have been reports of cilevirus, higrevirus, carmovirus, and tobamovirus infecting hibiscus in Hawaii [2, 3].A potent method for virus discovery and detection, high-throughput sequencing (HTS) has seen widespread application in the past ten years [4]. Hhibiscus plants exhibiting probable viral symptoms were spotted on the University of Hawaii at Manoa campus in the spring of 2020; however, the symptoms differed slightly from those previously documented in Hawaii. HTS samples from these plants were used for additional research.

RESOURCES AND PROCEDURES

Gathering Plant Materials and Preparing Samples

On the University of Hawaii at Manoa campus (21₀18′07″ N, 157₀48′55″ W), Hibiscus rosa-sinensis with foliar mosaic, ringspot, and chlorotic spots (Figure 1) was observed. As were extracted from the symptomatic leaves using a Spectrum® Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA) in accordance with the manufacturer's protocol. Using a Nano0/2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), total RNAs were quantified and quality controlled. To create composite samples, three of the total RNA samples were equimolarly combined. Thermo Fisher Scientific Inc.'s RiboMinusTM Plant Kit was used to extract ribosomal RNAs (rRNAs) from the composite samples, and the Illumina TruSeq ® RNA Sample Prep Kit (Illumina, San Diego, CA, USA) was utilized to create a cDNA library from the rRNA-depleted RNA samples.

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Assembly of Genomes and HTS Sequencing

The cDNA libraries were subjected to HTS using an Illumina NextSeq 500 sequencer, which produced 75-bp paired-end reads. Utilizing Trimmomatic® (Galaxy Version 0.38.0) to eliminate low-quality and adapter sequences, raw reads were then used with Trinity® (Galaxy Version 2.9.1+galaxy2) for de novo assembly into contigs. The sequences that matched reference sequences of viruses and viroids were subsequently found by querying contigs using BLASTx/BLASTn accessed on 25 October 2022). Rapid amplification of cDNA ends (RACE) was used to extract the 5′- and 3′-end sequences of the RNA viral genomes. The SMARTer®RACE 5′/3′ Kit (Takara, San Jose, CA, USA) was used in accordance with the manufacturer's instructions. A pRACE vector (Promega, Madison, WI, USA) was used to either directly sequence or clone all amplicons, and at least three separate clones were sequenced. Following that, complete viral sequences from HTS and RACE that resembled betacarmoviruses and soymoviruses were used as reference sequences utilizing raw reads and the Bowtie2 mapper (Version 2.4.5) plug-in, which is integrated into Geneious®Prime (Version 2022.2.1).

Virus Survey and RT-PCR Detection

Contig-specific primer sequences were created using Primer3 and BLASTn and used in RT-PCR assays to validate HTS and confirm the presence of individual viruses (Table 1). All PCR reactions used the GoTaq® Green Master Mix (Promega) and random primers to reverse transcribe total RNAs into cDNAs. RT-PCR products were resolved on 1.5% agarose gels stained with ethidium bromide and visualized under UV light. Amplicons were either sequenced directly or first ligated into a linearized pRACE vector (Takara).Three unique clones of each PCR result with inserts of the predicted sizes were then selected and sequenced in both directions by Sanger sequencing (Genewiz, San Diego, CA, USA). An additional 54 symptomatic and asymptomatic leaves from various Hibiscus spp. were sampled from other locations on Oahu.

Bioinformatic and Phylogenetic Examinations

The genome sequences produced in this study using Geneious® Prime (Version 2022.2.1) were aligned with whole genome sequences from homologs of pertinent reference viruses that were retrieved from GenBank and MUSCLE for phylogenetic analyses. A K2 + G + I substitution model and a maximum likelihood algorithm were used to create a phylogenetically oriented tree in MEGA X [5]. One thousand bootstrap iterations provided statistical support for groups inside the trees. Geneious® Prime (Version 2020.2.4) was also utilized for bioinfor- matic analysis of the genome sequences, including identification of ORFs, prediction of encoded proteins, pairwise sequence comparisons of nucleotide and protein amino acids, and sequence alignments.

Inoculation by Mechanical Means

Using carborundum and extracts from hibiscus plants infected with hibiscus betacarmovirus and hibiscus soymovirus, sixteen Nicotiana benthamiana plants and five Nicotiana tabacum plants were mechanically inoculated; three plants were mock-inoculated. The extracts were made by homogenizing plant leaf tissue with sodium phosphate buffer (20 mM sodium phosphate, pH 7.0) at a ratio of 1:10 (w/v).

RESULTS

Analysis of HTS Data

Following quality control, trimming, and de novo assembly, approximately 77.4 M paired-end reads were generated, and 162,000 contigs were obtained, as described in Section 2.2. Contig annotation using BLASTX/BLASTN against the viral database in GenBank revealed the presence of two viruses: a soymovirus that we called hibiscus soymovirus (HSV) and a betacarmovirus that we called hibiscus betacarmovirus (HBCV). Of ~76.8 M HTS trimmed reads, 8,721,585 (11.35%) and 7717 (0.01%) reads were mapped onto the complete genome sequences of hibiscus HBCV and HSV, respectively. The three samples used for HTS were also subjected to individual RT-PCR detection and tested positive for both viruses.

Discovery of Hibiscus Betacarmovirus (HBCV), a Novel RNA Virus

HBCV shared the highest whole genome nucleotide sequence identity (68.4%) with a hibiscus chlorotic ringspot virus (HCRSV) Israel isolate (KC876666) and the highest whole genome amino acid sequence identity (58.8%) with an HCRSV South Africa isolate (UUU45889). The HBCV genome contained three putative ORFs: HBCV ORF1 (nt 36–2276) encoded a putative viral coat protein (CP) of 356 aa, ORF2 (nt 2243–2464) encoded a putative viral movement protein (222 aa), and ORF3 (nt 2610–3677) encoded a putative viral coat protein (CP) of 356 aa.

Discovery of Hibiscus Soymovirus (HSV), a Novel DNA Virus

HTS was able to obtain the full genome sequence of the hibiscus soymovirus (HSV), which included 8143 nucleotides (GenBank accession number OP757659). The HSV genome contained ten putative ORFs and their locations: ORF1 (nt 45–296), ORF2 (nt 372–800), ORF3 (nt 797–1129), ORF4 (nt 1131–1298), ORF5 (nt 1298–2710), ORF6 (nt 2703–4760), ORF7 (nt 4739–5005), ORF8 (nt 5319–6125), ORF9 (nt 6769–7197), and ORF10 (nt 7190–8095), in that order. With the conserved domains of ribonuclease H (RNase H) and reverse transcriptase (RT), HSV ORF6 encoded a putative viral replicase.

Survey of Viruses

To ascertain the prevalence, range, and variety of these viruses in Hawaii, more hibiscus leaf samples from other areas were analyzed. One sample tested positive for HBCV, nine samples tested positive for HSV, and two samples tested positive for both HBCV and HSV. Of the fifty-four samples tested, twelve (22%) tested positive for at least one virus.

Inoculation by Mechanical Means

Through mechanical inoculation, we attempted to transfer the two viruses from hibiscus plants to 16 N. benthamiana and 5 N. tabacum plants. Six weeks after inoculation, the newest fully expanded leaf of each plant was examined using RT-PCR using the particular primers mentioned above to confirm viral infection. For RT-PCR, the RNA used for HTS served as a positive control. According to the RT-PCR results, the RNA samples from these inoculated plants and the no template control did not have a band of the expected size, whereas the positive control did. HBCV and HSV could not be mechanically inoculated into either N. benthamiana or N. tabacum using the current protocol, according to the results of the mechanical inoculation experiments and RT-PCR assays.

DISCUSSION

Less than 57% RdRp amino acid sequence identity and less than 52% CP amino acid sequence identity are required to define a species in the genus Betacarmovirus [2].Table 2 shows that the amino acid sequence of HBCV's RdRp was most similar to that of the HCRSV Israel isolate in terms of identity (53.3%), while the amino acid sequence of HBCV's CP was most similar to that of the HCRSV Singapore isolate in terms of identity (34.0%).These findings implied that HBCV is a member of a novel carmovirus species.Due to the complexity of its phylogenetic tree, the once-largest genus in the family Tombusviridae, Carmovirus, has been split into three new genera: Alphacarmovirus, Betacarmovirus, and Gammacarmovirus [6, 7]. A phylogenetic tree was created using the genomic nucleotide sequences of HBCV and the members of these three genera. HBCV was assigned to the Betacarmovirus clade, which is closely related to HCRSV, by the phylogenetic analysis (Figure 2). Therefore, we suggest classifying HBCV under the Betacarmovirus HBCV species.

A difference of more than 20% in polymerase (RT + RNase H) nt sequences is the criterion used to distinguish species in the genus Soymovirus [8]. The HSV polymerase's nucleotide sequence was the most similar (71.1%) to that of the polymerase to the peanut chlorotic streak caulimovirus (U13988), a soymovirus. These findings imply that HSV is a member of a novel virus species in the genus Soymovirus, which we suggest we call Soymovirus HSV.There are currently only four species of soymoviruses, which are single-component double-stranded DNA viruses along with two suspected additional species [9,10].According to the phylogenetic analysis, HSV and peanut chlorotic streak virus are most similar.Two novel viruses that infect hibiscus were found in this study, and their symptoms differed from those previously documented in Hawaii. This could be a novel plant virus disease that has surfaced on Hawaii's hibiscus.

REFERENCES

1. Mall, S.; Pandey, V.; Srivastava, A.; Gaur, R.K. Detection and characterization of plant viruses infecting hibiscus rosa-sinensis L. In Virus Diseases of Ornamental Plants; Springer: Berlin/Heidelberg, Germany, 2021; pp. 151–164. [CrossRef].

2. Melzer, M.J.; Simbajon, N.; Carillo, J.; Borth, W.B.; Freitas-Astúa, J.; Kitajima, E.W.; Neupane, K.R.; Hu, J.S. A cilevirus infects ornamental hibiscus in Hawaii. Arch. Virol. 2013, 158, 2421–2424. [CrossRef] [PubMed].

3. Olmedo-Velarde, A.; Hu, J.; Melzer, M.J. A virus infecting hibiscus rosa-sinensis represents an evolutionary link between cileviruses and higreviruses. Front. Microbiol. 2021, 12, 660237. [CrossRef] [PubMed].

4. Villamor, D.E.V.; Ho, T.; Al Rwahnih, M.; Martin, R.R.; Tzanetakis, I.E. High throughput sequencing for plant virus detection and discovery. Phytopathology 2019, 109, 716–725. [CrossRef] [PubMed].

5. Kumar, S.; Stecher, G.; Tamura, K. Molecular evolutionary genetics analysis (MEGA) for macOS. Mol. Biol. Evol. 2020, 37, 1237–1239. [CrossRef].

6. Adams, M.J.; Lefkowitz, E.J.; King, A.M.Q.; Harrach, B.; Harrison, R.L.; Knowles, N.J.; Kropinski, A.M.; Krupovic, M.; Kuhn, J.H.; Mushegian, A.R.; et al. Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2016). Arch. Virol. 2016, 161, 2921–2949. [CrossRef] [PubMed].

7. Scheets, K.; White, K.A.; Rubino, L.; Martelli, G.; Rochon, D.A. ICTV Taxonomic proposal 2015.007a-rP.v1.split_ Carmovirus. Divide the genus Carmovirus (family Tombusviridae) into three new genera: Alphacarmovirus, Betacarmovirus, and Gammacar- movirus.

8. King, A.M.Q.; Adams, M.J.; Carstens, E.B.; Lefkowitz, E. Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses; Elsevier: Amsterdam, The Netherlands, 2011; pp. 435–436.