Cytogenetic and Genome Research

Short Report

Cytogenet Genome Res 2020;160:329–334
DOI: 10.1159/000509845

Received: January 27, 2020
Accepted: June 22, 2020
Published online: July 18, 2020

Evidence of Rubus Yellow Net Virus Integration into the Red Raspberry Genome

Alfredo Diaz-Lara[a] Nola J. Mosier[b] Kristian Stevens[c] Karen E. Keller[b] Robert R. Martin[b]

[a]Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR, USA and Department of Plant Pathology, University of California-Davis, Davis, CA, USA; [b]USDA-ARS, Horticultural Crops Research Unit, Corvallis, OR, USA; [c]Department of Evolution and Ecology, University of California-Davis, Davis, CA, USA

Keywords
Discrimination · Genome · Integrated · Pararetrovirus · *Rubus* spp.

Abstract
Rubus yellow net virus (RYNV) infects *Rubus* spp., causing a severe decline when present in mixed infections with other viruses. RYNV belongs to the family *Caulimoviridae*, also known as plant pararetroviruses, which can exist as episomal or integrated elements (endogenous). Most of integrated pararetroviruses are noninfectious; however, a few cases have been reported where they excised from the plant genome and formed infectious particles. Graft transmission onto indicator plants *R. occidentalis* "Munger" has been the standard test method for RYNV detection in certification programs. Previously, it was noticed that some RYNV PCR-positive plants did not induce symptoms on "Munger", suggesting an integration event. In this study, bio-indexing and different molecular techniques were employed to differentiate between integrated and episomal RYNV sequences. Reverse transcription-PCR using RYNV-specific oligonucleotides after DNase treatment generated positive results for the virus in graft transmissible isolates (episomal) only. To confirm these results, rolling circle amplification on DNA preparations from the same samples resulted in amplicons identified as RYNV only from plants with graft transmissible RYNV. High-throughput sequencing was used to identify the RYNV-like sequences present in the host DNA. These results indicate the integration of RYNV into the red raspberry genome and highlight the necessity to recognize this phenomenon (integration) in future *Rubus* quarantine and certification programs.

Raspberries are classified in the genus *Rubus* (family Rosaceae), which includes hundreds of species and hybrid berry cultivars [Martin et al., 2013]. Approximately 6,839 hectares of red raspberry (*R. idaeus*) and black raspberry (*R. occidentalis*) were harvested in the USA in 2018 with a farm gate value of approximately 450 million US dollars (http://www.fao.org/faostat), making this an economically important crop. Raspberry is susceptible to numerous diseases, including those caused by viruses; there are reports of more than 30 viruses or virus-like diseases affecting *Rubus* spp. [for review, see Martin et al., 2013].

Rubus spp. are hosts of Rubus yellow net virus (RYNV), whose symptoms on plants range from net-like chlorosis of the tissue along the veins to asymptom-

karger@karger.com
www.karger.com/cgr

Alfredo Diaz-Lara
Department of Plant Pathology, University of California-Davis
One Shields Avenue
Davis, CA 95616-8751 (USA)
E-Mail adiazlara@ucdavis.edu

atic [Stace-Smith and Jones, 1987]. RYNV in mixed infections with raspberry leaf mottle virus and black raspberry necrosis virus cause raspberry mosaic disease, a disorder that severely affects plant vigor and yield [Jones et al., 2002]. RYNV produces bacilliform particles (virions) containing a circular double-stranded DNA genome of 7.9 kb and encodes a large polyprotein [Jones et al., 2002; Kalischuk et al., 2013; Diaz-Lara et al., 2015], features typical of the genus *Badnavirus*, family *Caulimoviridae*.

Caulimoviruses or plant pararetroviruses replicate via a cellular RNA intermediate (reverse transcription step) without integrating into the host genome, as in the case of animal retroviruses [Lyttle et al., 2011]. Despite their nonintegrative replication cycle, there is evidence that several pararetroviruses can exist as episomal and integrated forms [Harper et al., 2002; Gilbert and Feschotte, 2018]. Episomal viruses produce virions capable of causing infection in the host, on the other hand, endogenous pararetroviruses (EPRVs) are integrated in the host genome and have been reported among the plant kingdom [Staginnus and Richert-Pöggeler, 2006].

Over the past few years, several red raspberry plants that indexed negative for RYNV in aphid or graft transmission assays onto the biological indicator *R. occidentalis* "Munger" gave positive results in PCR-based tests, leading to rejection of plants by quarantine personnel in importing countries. In this work, we investigated the possibility that RYNV is integrated into the genome of selected red raspberry cultivars using bio-indexing, reverse transcription (RT)-PCR, rolling circle amplification (RCA) and high-throughput sequencing (HTS).

Materials and Methods

Plant Material and Initial PCR Test

Seven different red raspberry cultivars ("Caroline", "Comox", "Glen Clova", "Meeker", "Willamette", "Cascade Bounty," and "Baumforth's Seedling A") were obtained from the National Clonal Germplasm Repository and the Horticultural Crops Research Unit (HCRU) of the USDA-ARS in Corvallis, Oregon. The previously mentioned plants were selected based on an initial test, in which they tested positive for RYNV using the PCR primers RYNV6-F (5′-CGTGATAACGGTTTGGTTTT-3′) and RYNV6-R (5′-CGTAAGCGCAGATTTCTTCC-3′) that target the viral reverse transcriptase and ribonuclease H domains, respectively. PCR amplification was performed using *Taq* polymerase (GenScript, Piscataway, NJ, USA; PCR program consisted of 3 min at 95°C; 40 cycles of 40 s at 95°C, 40 s at 56°C, and 40 s at 72°C, with a final elongation step of 10 min at 72°C) and yielded amplicons of about 400 bp. Direct sequencing confirmed that the amplicons represented RYNV.

Bio-Indexing

The 7 red raspberry cultivars were employed as donor sources for graft transmission onto "Munger". The "Munger" plants were obtained from a commercial nursery in Oregon and were propagated from plants free of known viruses. In addition, all the "Munger" plants were tested with the RYNV6-primers to confirm their RYNV-free status before initiation of the experiment. Three repetitions per donor plant were used, and each indicator plant was grafted twice (2 grafts). Lastly, the grafted plants were maintained in a greenhouse at the USDA-ARS HCRU at 25°C with 16 h daylight, and the plants were evaluated for RYNV symptoms 3, 6, and 8 weeks post-grafting as well as tested for RYNV (RYNV6 PCR) at the end of the observation period. The expected symptoms on "Munger" were uneven growth of the basal leaflets of the trifoliates and vein chlorosis that give a yellow-netted appearance to the leaves [Martin et al., 2013].

Nucleic Acids Extraction

Different nucleic acid extraction protocols were used for the following molecular tests. In the case of RT-PCR, the protocol described by Quito-Avila and Martin [2012] was employed to obtain total RNA from red raspberry leaves. For RCA, total DNA was extracted from plant material as previously described by Diaz-Lara et al. [2015]. Finally, the FastDNA SPIN Kit and the FastPrep Instrument (MP Biomedicals, Santa Ana, CA, USA) were utilized, according to manufacturer's recommendations to isolate high-quality genomic DNA for downstream HTS.

RT-PCR and RCA

The red raspberry cultivars were analyzed by RT-PCR and RCA to determine whether RYNV is actively replicating or is integrated in the genome of the host plant. RNA extracts from the samples were digested with 1 μL of RQ1 RNase-Free DNase (1U/L) (Promega, Madison, WI, USA) following the manufacturer's recommendations prior to RT-PCR. 2.5 μL of DNase-digested product was used as template for RT using Maxima Reverse Transcriptase (Thermo Fisher, Hudson, NH, USA) and the RT reaction was incubated for 60 min at 50°C, then 5 min at 85°C, followed by PCR using the RYNV6 primers as described previously. In parallel with the RYNV testing, an assay [Tzanetakis et al., 2007; Thekke-Veetil et al., 2016] targeting the mRNA and/or the genomic copy of NADH dehydrogenase ND2 subunit (*ndhB* gene) was used to assess the DNA digestion and consequently the presence of undigested DNA, potential for false positives during the RT-PCR test.

One μg of sample DNA was treated with 10 units of Exonuclease V (New England Biolabs, Ipswich, MA, USA) at 37°C for 30 min before RCA to eliminate the linear DNA, potential interference with the RCA reaction, without affecting the circular DNA. The RCA was carried out using the Illustra TempliPhi 500 Amplification Kit (GE Healthcare, Buckinghamshire, UK) following the manufacture's protocol, but adding the RYNV6-F primer to the reaction. Finally, the RCA product was digested with *Bam*HI (New England Biolabs) and then size analyzed by electrophoresis (1.5% agarose gel). The RYNV genome contains only one *Bam*HI recognition site.

High-Throughput Sequencing and PCR Validation

To characterize the RYNV-like sequences present in the red raspberry genome, genomic DNA from the 7 cultivars involved in this study was sequenced using the Illumina HiSeq 3000 platform

(Illumina, San Diego, CA, USA), using a paired-end (2 × 150 bp) configuration. Briefly, DNA samples were subjected to library construction using a TruSeq DNA HT Sample Prep Kit (Illumina) and sequenced individually, 1 cultivar per lane of a flow cell. Later, for each library, paired-end sequences were assembled using MEGAHIT [Li et al., 2015] with default parameters. Assemblies (contigs) were subsequently formatted as a searchable BLAST database for virus annotation. A BLASTn search was performed against each assembly using the GenBank entry NC026238 (RYNV reference genome) as the query. A custom script was used to extract assembled contigs where the virus sequence only aligned partially with the target RYNV sequence and included 5′ or 3′ overhanging ends. The remaining unaligned portion of the contig, potentially host genome, was considered for downstream validation.

Studied plants were analyzed by PCR using sets of primers that amplify 3 putative virus-plant junctions: (a) Baumforth_k99_4826083_F (5′-GTGAGTACCACTTCAATGAGTA-3′ and Baumforth_k99_4826083_R (5′-TAGTTGCCTCTGCTCCTTTA-3′); (b) Caroline_k99_1872834_F (5′-GTACTGCTTCTTCTCGTAGTAT-3′) and Caroline_k99_1872834_R (5′-GTGGTACTTGATTTGGTTGTAG-3′, and (c) Cascade_k99_7189637_F (5′-AGAAAGCTTATAGTACCGTGAG-3′) and Cascade_k99_7189637_R (5′-CAGAATTATCAAGCACGGAAG-3′). The previous junctions were selected based on essential characteristics for primer design, GC content and length; thus, 1 primer was located at the RYNV-like sequence, and the complement primer targeted the predicted plant sequence. To determine whether junctions are consistent among plants, all the 7 red raspberry cultivars were individually tested by the designed primers. Finally, the amplified products were directly sequenced in both directions.

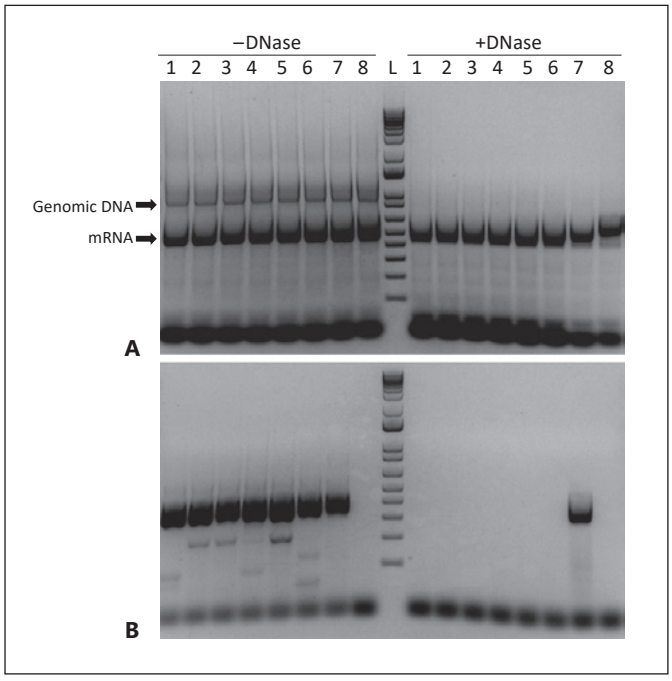

Fig. 1. Agarose gel electrophoresis of RT-PCR products. **A** Assay for the NADH dehydrogenase ND2 subunit (*ndhB* gene). **B** Assay for Rubus yellow net virus (RYNV) using the RYNV6F/RYNV6R oligonucleotide primers. –DNase, total RNA extracts were used as template for RT-PCR without prior digestion; +DNase, total RNA extracts were DNase digested before RT-PCR. Lanes 1–7 represent different red raspberry cultivars: 1, "Caroline"; 2, "Comox"; 3, "Glen Clova"; 4, "Meeker"; 5, "Willamette"; 6, "Cascade Bounty"; 7, "Baumforth's Seedling A". Lane 8 represents black raspberry (negative control). Lane L represents 1 kb Plus DNA Ladder.

Results

Bio-Indexing

Indicator plants grafted with "Baumforth's Seedling A" started to show RYNV-symptoms (vein chlorosis) 6 weeks post-grafting. On the other hand, the rest of the red raspberry cultivars tested failed to induce symptoms after graft transmission on "Munger." Additionally, only the "Munger" plants grafted with "Baumforth's Seedling A" tested positive by the RYNV6 PCR assay.

RT-PCR and RCA

Except for the cultivar "Baumforth's Seedling A", the rest of the samples ("Caroline", "Comox", "Glen Clova", "Meeker", "Willamette," and "Cascade Bounty") was negative for RYNV after the RT-PCR test with the additional DNase-treatment (Fig. 1B). On the other hand, all the samples were positive for the virus via RT-PCR when samples were not digested with DNase prior to RT-PCR, suggesting that the amplification originated from DNA rather than RNA in the cultivars other than "Baumforth's Seedling A." In that sense, the amplification of 2 different bands by the *ndhB* gene assay using not DNase-digested template, contrast with the single amplicon generated from the digested template (Fig. 1A); this indicates the absence of genomic DNA after the DNase treatment. Supporting the RT-PCR results, RCA yielded an amplicon from the sample "Baumforth's Seedling A," but not from the other red raspberry cultivars in the study (data not shown). A DNA band of nearly 8 kb in size was observed after the RCA product was treated with *Bam*HI; the RCA product matched the predicted size of RYNV.

HTS and PCR Validation

The HTS analysis resulted in around 300 million paired reads per DNA library (cultivar). Based on the BLAST analysis, 25 contigs were found to share 77–90% nucleotide sequence identity with the RYNV reference genome (Table 1). The size of such contigs was between 246 and 3,259 nucleotides, overhanging with the predict-

Table 1. Assembly contigs where Rubus yellow net virus sequence aligned partially

Assembly	5′ overhang nucleotides	3′ overhang nucleotides	Identity[a], %	Qlen	Qstart	Qend	Clen	Cstart	Cend	E-value[a]
Baumforth_k99_4826083	433	0	77	7,836	1	1,877	2,308	434	2,308	0
Baumforth_k99_1746789	0	15	85	7,836	3,566	4,421	871	1	856	0
Baumforth_k99_11970	13	9	87	7,836	141	763	644	14	635	0
Baumforth_k99_4175922	0	17	84	7,836	815	1,268	471	1	454	1.41E-122
Caroline_k99_1872834	8	1,669	82	7,836	3,286	4,870	3,259	1,590	9	0
Caroline_k99_4068957	13	0	88	7,836	141	416	288	14	288	1.69E-86
Caroline_k99_11066072	267	0	80	7,836	12	281	541	268	541	3.84E-48
Caroline_k99_10199738	12	7	78	7,836	7,551	7,830	301	294	13	3.87E-43
Caroline_k99_4929383	0	234	90	7,836	191	281	325	91	1	3.08E-24
Cascade_k99_10532002	0	15	85	7,836	3,566	4,421	871	1	856	0
Cascade_k99_9699367	1,614	6	77	7,836	1	1,118	2,734	1,615	2,728	1.13E-173
Cascade_k99_7189637	0	284	80	7,836	12	281	558	274	1	4.83E-48
Cascade_k99_9349915	12	7	79	7,836	7,551	7,830	301	294	13	1.05E-44
Cascade_k99_10596469	0	234	90	7,836	191	281	325	91	1	3.87E-24
Comox_k99_4640982	18	0	86	7,836	916	1,175	278	278	19	3.65E-73
Comox_k99_3670332	0	15	84	7,836	4,141	4,421	296	1	281	1.70E-71
Comox_k99_2182286	12	7	79	7,836	7,551	7,830	301	294	13	8.19E-45
Comox_k99_2140863	27	1	80	7,836	1,557	1,774	246	28	245	1.78E-36
Glenclova_k99_2830577	0	15	85	7,836	3,566	4,421	871	1	856	0
Glenclova_k99_8263433	22	0	81	7,836	1,674	2,575	915	915	23	0
Meeker_k99_1270087	0	15	85	7,836	3,566	4,421	871	1	856	0
Meeker_k99_8020345	12	7	79	7,836	7,551	7,830	301	294	13	8.01E-45
Willamette_k99_5753313	0	15	85	7,836	3,566	4,421	871	1	856	0
Willamette_k99_6330268	0	28	84	7,836	2,852	3,208	379	1	351	2.19E-90
Willamette_k99_8644630	12	7	79	7,836	7,551	7,830	301	294	13	8.41E-45

[a] BLASTn analysis. Qlen, query length; Qstart, query start; Qend, query end; Clen, contig length, Cstart, contig start; Cend, contig end.

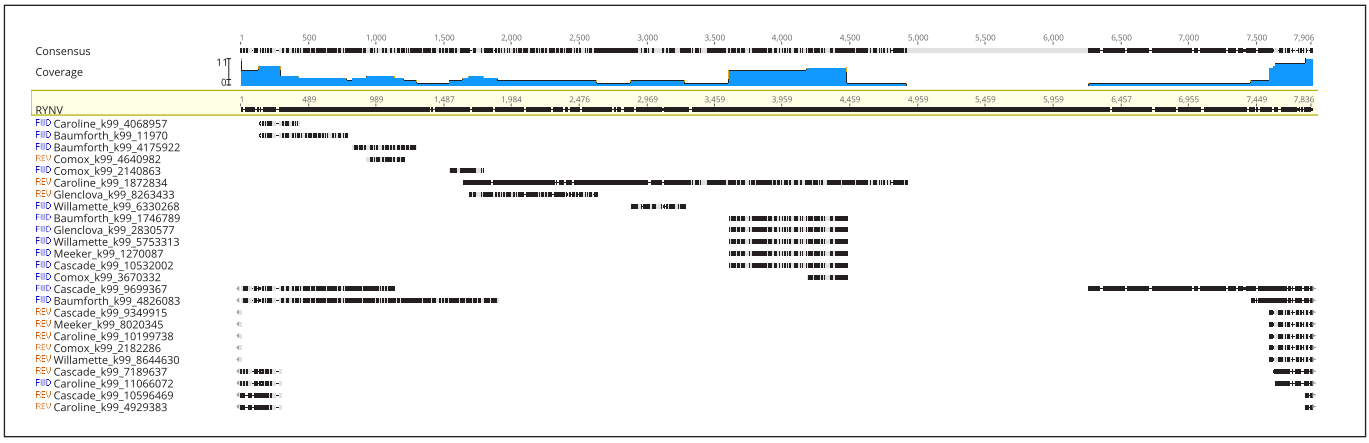

Fig. 2. Contigs mapped against Rubus yellow net virus (RYNV) genome. The contig name (left) includes the plant source. Blue section (top) denotes the grade of coverage between the reference sequence (RYNV) and the different contigs. FWD, forward sequence; REV, reverse sequence. Contigs were mapped using Geneious v10.2.

ed plant genome in one or both ends (5′ and 3′). Mapping all the RYNV-like contigs from the different cultivars, not a full coverage of the RYNV genome was obtained (Fig. 2). From the pool of contigs, 3 contigs (Baumforth_k99_4826083, Caroline_k99_1872834 and Cascade_k99_7189637; GenBank: MT318147–MT318149) were subjected to PCR validation and Sanger sequencing. As a result, each of these 3 contigs were amplified from the source plants and mapped well to the original sequence; Cascade_k99_7189637 contig was not only amplified from the source plant, but also from the "Caroline" cultivar.

Discussion

The work presented here clearly indicates that RYNV is integrated into the genome of each of the 7 red raspberry cultivars studied. Multiple molecular techniques and bio-indexing were used to differentiate between episomal and integrated sequences of RYNV in several red raspberry cultivars. In consequence, RYNV-like sequences in the red raspberry genome should be referred as endogenous RYNV-like sequences (eRYNVs).

Graft indexing is a popular technique for virus detection, especially in the case of unknown viruses; however, in this study, we used grafting for a different reason: to determine whether or not red raspberry cultivars hosted a form of RYNV with infectious capacity. In the case of the RT-PCR assay, the aim of including a DNase digestion before the RT-PCR was to remove any RYNV-like sequence present in the genomic DNA of red raspberry, thus targeting only RNA transcripts of episomal viruses. Similar methodology was employed in a study reporting a new badnavirus in blackberry with integration capacity [Shahid et al., 2017]. RYNV belongs to the family Caulimoviridae, and the RCA amplifies the circular RYNV genome and not the endogenous virus, since it is not a circular DNA. Overall, only the "Baumforth's Seedling A" plant validated the presence of an episomal RYNV with infectious/replication capacity.

There are previous reports of integrated pararetrovirus sequences with nearly perfect identity to episomal viruses in plants. For example, EPRVs have been reported from banana [Harper et al., 1999], tobacco [Gregor et al., 2004], petunia [Richert-Pöggeler et al., 2003], rice [Kunii et al., 2004], fig [Laney et al., 2012], yam [Umber et al., 2014], blackberry [Shahid et al., 2017], and citrus [Yu et al., 2019]. Most of the integrated virus-like sequences were rearranged in the host DNA, suggesting illegitimate recombination [Harper et al., 2002]; such recombination may have occurred when the episomal virus was replicating in the host [Kunii et al., 2004]. Recently, EPRVs have been used to study the heterogeneity of viral gene macroevolution [Chen et al., 2018].

Based on the HTS result, all the red raspberry cultivars included in this study contain in their genetic material sequences with high homology to RYNV, including the plant carrying the graft transmissible isolate of RYNV ("Baumforth's Seedling A"). Such RYNV-like sequences were identified as assembly contigs that mapped partially to the virus genome and partially to the *Rubus* genome, presumably originated from virus-plant junctions, and a complete RYNV genome was not identified. Furthermore, assemblies mapped in different regions of the RYNV genome (Fig. 2), except for the predicted region of the replication-associated domains, which in combination with the PCR validation suggests that identified eRYNVs are independent inserts or not conserved.

The de novo genome assemblies should offer a limited but complete picture of virus integration in the red raspberry genome. Each assembly gives a fragmented but hopefully complete picture of the nonrepetitive parts of the plant genome. The use of a detailed red raspberry genome, not publicly available, as reference would improve the identification of eRYNVs and reveal the specific location of integrations in the chromosomes. Lastly, the use of FISH may result in the precise identification of eRYNVs.

Additionally, discerning the structure and prevalence of the integrated elements is important, as some EPRVs have been reported to activate, assembly of virions and induce infection, after exposure to abiotic stress or tissue culture [Ndowora et al., 1999; Richert-Pöggeler et al., 2003; Kuriyama et al., 2020]. In the case of integrated sequences of banana streak virus, the process of propagating plants through tissue culture resulted in excision from the banana genome to cause infection [Harper et al., 2002]. Although the process of integration into and excision from the red raspberry genome is beyond the goal of this study, during an independent work, plants with integrated RYNV sequences were subjected to repeated heat therapy, meristem tip-culture and plant regeneration in tissue culture, and there were no examples of RYNV excision observed.

From a genomic perspective, the presence of eRYNVs in different red raspberry cultivars and potentially in their offspring makes their elimination difficult; however, new genome editing tools such as CRISPR-Cas could provide tools to remove the eRYNVs [Tripathi et al., 2019]. Thus,

the ability to differentiate between episomal and integrated RYNVs can avoid unnecessary costs of false positives at virus-elimination stages, and more importantly, restriction on plant movement based on inaccurate virus diagnosis.

Acknowledgment

We thank Jake Dittrich (Oregon State University) for his technical support during this study. We also thank Joseph Postman (National Clonal Germplasm Repository-USDA) for providing plant material.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the USDA-ARS and the National Clean Plant Network.

Author Contributions

A.D.-L.: Conceptualization, methodology, validation, formal analysis, investigation, data curation. N.J.M.: Conceptualization, methodology, validation, investigation. K.S.: methodology, formal analysis, data curation. K.E.K.: formal analysis, investigation, data curation. R.R.M.: Conceptualization, methodology, formal analysis, investigation.

All authors participated in writing and editing the manuscript.

References

Chen S, Saito N, Encabo JR, Yamada K, Choi IR, Kishima Y: Ancient endogenous pararetroviruses in *Oryza* genomes provide insights into the heterogeneity of viral gene macroevolution. Genome Biol Evol 10:2686–2696 (2018).

Diaz-Lara A, Mosier NJ, Keller KE, Martin RR: A variant of *Rubus yellow net virus* with altered genomic organization. Virus Genes 50:104–110 (2015).

Gilbert C, Feschotte C: Horizontal acquisition of transposable elements and viral sequences: patterns and consequences. Curr Opin Genet Dev 49:15–24 (2018).

Gregor W, Mette MF, Staginnus C, Matzke MA, Matzke AJM: A distinct endogenous pararetrovirus family in *Nicotiana tomentosiformis*, a diploid progenitor of polyploid tobacco. Plant Physiol 134:1191–1199 (2004).

Harper G, Osuji JO, Heslop-Harrison JS, Hull R: Integration of Banana streak Badnavirus into the *Musa* genome: molecular and cytogenetic evidence. Virology 255:207–213 (1999).

Harper G, Hull R, Lockhart B, Olszewski N: Viral sequences integrated into plant genomes. Annu Rev Phytopathol 40:119–136 (2002).

Jones AT, McGavin WJ, Geering ADW, Lockhart BEL: Identification of *Rubus yellow net virus* as a distinct badnavirus and its detection by PCR in *Rubus* species and in aphids. Ann Appl Biol 141:1–10 (2002).

Kalischuk ML, Fusaro AF, Waterhouse PM, Pappu HR, Kawchuk LM: Complete genomic sequence of a *Rubus yellow net virus* isolate and detection of genome-wide pararetrovirus-derived small RNAs. Virus Res 178:306–313 (2013).

Kunii M, Kanda M, Nagano H, Uyeda I, Kishima Y, Sano Y: Reconstruction of putative DNA virus from endogenous Rice tungro bacilliform virus-like sequences in the rice genome: implications for integration and evolution. BMC Genomics 5:80 (2004).

Kuriyama K, Tabara M, Moriyama H, Kanazawa A, Koiwa H, et al: Disturbance of floral color pattern by activation of an endogenous pararetrovirus, petunia vein clearing virus, in aged petunia plants. Plant J, E-pub ahead of print (2020).

Laney AG, Hassan M, Tzanetakis IE: An integrated badnavirus is prevalent in fig germplasm. Phytopathology 102:1182–1189 (2012).

Li D, Liu CM, Luo R, Sadakane K, Lam TW: MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31:1674–1676 (2015).

Lyttle DJ, Orlovich DA, Guy PL: Detection and analysis of endogenous badnaviruses in the New Zealand flora. AoB Plants 2011:plr008 (2011).

Martin RR, MacFarlane S, Sabanadzovic S, Quito D, Poudel B, Tzanetakis IE: Viruses and virus diseases of *Rubus*. Plant Dis 97:168–182 (2013).

Ndowora T, Dahal G, LaFleur D, Harper G, Hull R, et al: Evidence that badnavirus infection in *Musa* can originate from integrated pararetroviral sequences. Virology 255:214–220 (1999).

Quito-Avila DF, Martin RR: Real-time RT-PCR for detection of *Raspberry bushy dwarf virus*, *Raspberry leaf mottle virus* and characterizing synergistic interactions in mixed infections. J Virol Methods 179:38–44 (2012).

Richert-Pöggeler KR, Noreen F, Schwarzacher T, Harper G, Hohn T: Induction of infectious *Petunia vein clearing* (pararetro) *virus* from endogenous provirus in petunia. EMBO J 22:4836–4845 (2003).

Shahid MS, Aboughanem-Sabanadzovic N, Sabanadzovic S, Tzanetakis IE: Genomic characterization and population structure of a badnavirus infecting blackberry. Plant Dis 101:110–115 (2017).

Stace-Smith R, Jones AT: *Rubus* yellow net. Agric. Handb. – U. S. Dep. Agric. Comb. For. Pest Res. Dev. Program USA (1987).

Staginnus C, Richert-Pöggeler KR: Endogenous pararetroviruses: two-faced travelers in the plant genome. Trends Plant Sci 11:485–491 (2006).

Thekke-Veetil T, Ho T, Moyer C, Whitaker VM, Tzanetakis IE: Detection of strawberry necrotic shock virus using conventional and TaqMan® quantitative RT-PCR. J Virol Methods 235:176–81 (2016).

Tripathi JN, Ntui VO, Ron M, Muiruri SK, Britt A, Tripathi L: CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. Commun Biol 2:46 (2019).

Tzanetakis IE, Postman JD, Martin RR: Identification, detection and transmission of a new *Vitivirus* from *Mentha*. Arch Virol 152:2027–2033 (2007).

Umber M, Filloux D, Muller E, Laboureau N, Galzi S, et al: The genome of African yam (*Dioscorea cayenensis-rotundata* complex) hosts endogenous sequences from four distinct badnavirus species. Mol Plant Pathol 15:790–801 (2014).

Yu H, Wang X, Lu Z, Xu Y, Deng X, Xu Q: Endogenous pararetrovirus sequences are widely present in Citrinae genomes. Virus Res 262:48–53 (2019).