Virus Research 184 (2014) 87–92

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Short communication

Hardenbergia mosaic virus: Crossing the barrier between native and introduced plant species

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ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 17 February 2014
Accepted 18 February 2014
Available online 1 March 2014

Keywords:
Virus emergence
Agro-ecological interface
Indigenous virus
Whole genomes
Genetic diversity
Recombination

ABSTRACT

Hardenbergia mosaic virus (HarMV), genus *Potyvirus*, belongs to the bean common mosaic virus (BCMV) potyvirus lineage found only in Australia. The original host of HarMV, *Hardenbergia comptoniana*, family *Fabaceae*, is indigenous to the South-West Australian Floristic Region (SWAFR), where *Lupinus* spp. are grown as introduced grain legume crops, and exist as naturalised weeds. Two plants of *H. comptoniana* and one of *Lupinus cosentinii*, each with mosaic and leaf deformation symptoms, were sampled from a small patch of disturbed vegetation at an ancient ecosystem–recent agroecosystem interface. Potyvirus infection was detected in all three samples by ELISA and RT-PCR. After sequencing on an Illumina HiSeq 2000, three complete and two nearly complete HarMV genomes from *H. comptoniana* and one complete HarMV genome from *L. cosentinii* were obtained. Phylogenetic analysis which compared (i) the four new complete genomes with the three HarMV genomes on Genbank (two of which were identical), and (ii) coat protein (CP) genes from the six new genomes with the 38 HarMV CP sequences already on Genbank, revealed that three of the complete and one of the nearly complete new genomes were in HarMV clade I, one of the complete genomes in clade V and one nearly complete genome in clade VI. The complete HarMV genome from *L. cosentinii* differed by only eight nucleotides from one of the HarMV clade I genomes from a nearby *H. comptoniana* plant, with only one of these nucleotide changes being non-synonymous. Pairwise comparison between all the complete HarMV genomes revealed nucleotide identities ranging between 82.2% and 100%. Recombination analysis revealed evidence of two recombination events amongst the six complete genomes. This study provides the first report of HarMV naturally infecting *L. cosentinii* and the first example for the SWAFR of virus emergence from a native plant species to invade an introduced plant species.

Hardenbergia mosaic virus (HarMV) (genus *Potyvirus*, family *Potyviridae*) is a recently described virus from the native perennial legume *Hardenbergia comptoniana*, a plant species endemic to the South-West Australian Floristic Region (SWAFR) (Hopper and Gioia, 2004; Webster et al., 2007; Coutts et al., 2011; Wylie and Jones, 2011). The SWAFR is a species rich global diversity hot spot with around 8000 indigenous plant species where there was no plant cultivation until Europeans arrived in 1829 (Myers et al., 2000; Hopper and Gioia, 2004). Coat protein (CP) gene nucleotide (nt) sequencing placed 30 HarMV isolates into eight clades with up to 21% nt differences between sequences (Webster et al., 2007). A ninth clade was suggested after the CP sequences of six additional HarMV isolates were added and compared with the initial 30 (Coutts et al., 2011). When their complete genomes were sequenced using Illumina GAIIx technology, two HarMV isolates found co-infecting a single *H. comptoniana* plant differed by 18% at the nt level (Wylie and Jones, 2011). The high degree of nt diversity over a small geographic range demonstrated by HarMV is characteristic of viruses that co-evolved with native plants locally over a long period of time (Spetz et al., 2003; Webster et al., 2007; Coutts et al., 2011).

Potyviruses found in Australia fall into two groups, with roughly half of them being isolated from cultivated plants and found in other parts of the world. Potyviruses isolated from *Lupinus* spp. so far fall into this category. The other half constitute a potyvirus lineage found only in Australia which belongs to the bean common mosaic virus (BCMV) group (Gibbs et al., 2008; Coutts et al., 2011). Members of the Australian potyvirus lineage have only been

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http://dx.doi.org/10.1016/j.virusres.2014.02.012
0168-1702/

isolated from native plants or naturalised weed species introduced as potential pasture species, apart from passionfruit woodiness virus (PWV) from cultivated *Passiflora* spp. (Gibbs et al., 2008; Coutts et al., 2011). HarMV is the best studied member of this lineage. At the CP level, HarMV is most closely related to six other potyviruses found only in Australia: clitoria chlorosis virus (CliVY), hibbertia virus Y (HiVY), siratro 1 virus Y (S1VY), siratro 2 virus Y (S2VY), passiflora mosaic virus (PaMV) and PWV (Coutts et al., 2011).

Lupinus spp. were introduced to the SWAFR around the early 1900s where they were used as sheep feed initially, and later domesticated as grain legume crops for rotation with cereals (French et al., 2008). *L. angustifolius* (narrow-leafed lupin), *L. cosentinii* (sandplain lupin), *L. luteus* (yellow lupin) and *L. mutabilis* (pearl lupin) became infected by HarMV experimentally in a glasshouse environment (Webster et al., 2007). Also, naturally occurring aphids spread HarMV from introduced *H. comptoniana* infector plants to *L. angustifolius* plants growing in experimental field plots (Luo et al., 2011). However, except within these field plots, HarMV has not been found infecting any *Lupinus* spp. or other introduced plant species naturally. We therefore investigated an introduced lupin-*H. comptoniana* interface scenario involving an ancient ecosystem (i.e. native Australian plants) and a recent agroecosystem (i.e. introduced species) in the SWAFR (Webster et al., 2007; Jones, 2009; Alexander et al., 2014; Vincent et al., 2014). As such, we report the first detection of HarMV infecting *L. cosentinii* naturally, and the first example of an indigenous virus effectively crossing the barrier between native and introduced species in the SWAFR. We also present four new complete and two nearly complete HarMV genome sequences, including one complete sequence from *L. cosentinii* and the remaining sequences from *H. comptoniana*.

Leaf tissue from two *H. comptoniana* and one *L. cosentinii* plants all showing leaf mosaic and deformation symptoms, were collected at the agro-ecological interface from a patch (50 m × 5 m) of disturbed native vegetation surrounding experimental field plots at Medina near Perth, Western Australia (WA). Within the patch, the *L. cosentinii* plant sampled was growing as a naturalised weed in close proximity (4 m) to the sampled *H. comptoniana* plants.

The samples were tested with generic potyvirus monoclonal antibodies (Agdia, USA) using the antigen-coated indirect ELISA protocol of Torrance and Pead, 1986. Absorbance values (A_{405}) were regarded as positive when more than three times those of the healthy sap control. For testing by RT-PCR, total RNA was extracted using a Spectrum Plant Total RNA kit (Sigma-Aldrich, Australia) according to manufacturers' instructions. Reverse transcription was performed with Improm-II reverse transcriptase (Promega, Australia) using the random primers provided according to manufacturer's instructions. PCR was performed with the *GoTaq* green master mix (Promega, Australia). PCR primers for generic potyvirus identification were from Webster, 2008.

Total RNA from each potyvirus positive sample was sent to the Australian Genome Research Facility (AGRF) for library preparation and barcoding (24 samples per lane) before 100 bp paired-end sequencing on an Illumina HiSeq2000. For each sample, reads were first trimmed using CLC Genomics Workbench 6.5 (CLCGW) (CLC bio) with the quality scores limit set to 0.01, maximum number of ambiguities to two and removing any reads with <30 nt. Contigs were assembled using the de novo assembly function of CLCGW with automatic word size, automatic bubble size, minimum contig length 500, mismatch cost two, insertion cost three, deletion cost three, length fraction 0.5 and similarity fraction 0.9. Contigs were sorted by length and the longest subjected to a BLAST search (Altschul et al., 1990). In addition, for samples MD2 and MD3 reads were also imported into Geneious 6.1.6 (Biomatters) and provided with a HarMV reference sequence obtained from Genbank

Table 1
Hosts, symptoms and sequence data for three leaf samples from plants infected with hardenbergia mosaic virus.[a]

Plant/host ID	Leaf symptoms[b]	No. of reads obtained	No. of reads after trimming	No. of contigs produced (CLC[c])	Sample sequence ID	Accession number	Contig length (nt)	Average coverage	No. of reads mapped to contig of interest (CLC)	Reference sequence used for mapping (Geneious[c])	Length of consensus (nt)	Average coverage	Number of reads mapped to ref. sequence (Geneious)	Final sequence length (nt)[d]
1.*Hardenbergia comptoniana*	M, LD	17,616,843	17,182,534	1691	MD2	KJ152152	9672	5072	499,921	NC015394	9751	5354	522,746	9647
2.*Lupinus cosentinii*	M, LD	13,154,290	12,840,928	677	MD3	KJ152153	9695	3516	347,261	NC015394	9726	3612	352,433	9647
3.*H. comptoniana*	M, LD	12,538,704	12,239,175	1806	MD4-A	KJ152154	9648	2975	292,188	–	–	–	–	9564
					MD4-B	KJ152155	8975	194	17,738	–	–	–	–	8906
					MD4-C	KJ152156	9635	343	33,716	–	–	–	–	9659
					MD4-D	KJ152157	9600	298	29,120	–	–	–	–	9668

[a] Three symptomatic plant samples were collected from a disturbed patch of native vegetation at the agro-ecological interface. Samples were sequenced on an Illumina HiSeq2000.
[b] Coded symptom descriptions: M, mosaic; LD, leaf deformation.
[c] Programs used for assembly of data: CLC, CLC Genomics Workbench 6.5 (CLC bio); Geneious 6.1.6 (Biomatters).
[d] Final sequences for MD2 and MD3 consisted of the consensus between de novo assembly and the mapped consensus. Final sequences for MD4A-D consisted of their de novo contigs only.