Genome sequence and variation in the Australian native *Velvet tobacco mottle virus*

by

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- Thesis Summary -

Velvet tobacco mottle virus (VTMoV; genus Sobemovirus) infects *Nicotiana velutina* (Velvet tobacco), a native of the arid region of central Australia. In the field, the virus, mirid vector and native host plant together comprise a unique plant virus pathosystem which is well adapted to its ecological niche, and independent of anthropogenic influences. The purpose of this research was to describe the sequence variation amongst VTMoV isolates and relate this to ecological factors.

The full genome sequence of VTMoV was obtained using a genome walking strategy with both degenerate and specific primers. Sequence and genome organisation confirm that VTMoV is a unique sobemovirus and phylogenetic analysis groups it separately from other sequenced Australian sobemoviruses. This is consistent with the hypothesis that VTMoV is not a recently introduced sobemovirus, but rather a product of evolution within a unique Australian ecosystem, representing a novel plant virus lineage. The genome sequences of two isolates of VTMoV, K1 and R17 were compared and a limited amount of variation was observed between these isolates.

Sequence diversity was observed in the RNA dependent RNA polymerase (RdRp) gene from 15 isolates of the virus. Analysis determined mutations were limited to maintain protein function, which is indicative of purifying selection. In addition, the first evidence of recombination in the RdRp of a sobemovirus was detected in three VTMoV sequences.

Sequence variation associated with transmission of VTMoV by the mirid *Cyrtopeltis nicotianae* [Hemiptera; Miridae] was also investigated. Isolates K1 and R17 were serially passaged monthly either through obligatory mirid transmission or via mechanical inoculation. After two years, sequences were compared from two regions of the VTMoV genome associated with movement (open reading frames of protein P1 and the coat protein). Several different trends were observed in the sequences, but only one difference could be associated with the mode of transmission. The coat protein region sequence from mirid transmission had a higher mutation rate that sequence from the mechanical mode of transmission.

This thesis contains the first complete genome sequence of VTMoV. It describes natural variation amongst a range of VTMoV isolates, and assesses sequence variation in parts of the genome after long term mirid transmission. The sequence of VTMoV is discussed in the context of the unique nature of the virus and the evolutionary mechanisms that may have played a role the evolution of the virus.

- Statement -

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List of published works:

Arthur, K., S. Dogra and J. W. Randles (2010). "Complete nucleotide sequence of *Velvet tobacco mottle virus isolate K1*." Archives of Virology 155 (11): 1893-1896.

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The process of scientific discovery is, in effect, a continual flight from wonder.

Albert Einstein

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Dedicated to my grandfather,

Whose adventurous spirit encouraged me to dream

- List of Abbreviations -

Abbreviation guidelines of the *Journal of General Virology* were followed for this thesis.

CP- Coat protein

DIBA- Dot immunobinding assay

d.p.i – days post inoculation

gRNA- genomic RNA

LB- Luria broth

mg- milligrams

MP- Movement protein

nm-nanometres

sgRNA – subgenomic RNA

VPg- viral genome-linked protein

WINC – Waite Institute culture collection

Virus abbreviations

Names not in italics signify species yet to be officially recognised by the International Committee on Taxonomy of Viruses (ICTV)

BBTV- *Banana bunchy top virus*

BSSV- *Blueberry shoestring virus*

BYDV- *Barley yellow dwarf virus*

CMV- *Cucumber mosaic virus*

CoMV- *Cocksfoot mottle virus*

IYMV- Imperata yellow mottle virus

LTSV – *Lucerne transient streak virus*

PLRV- *Potato leaf roll virus*

PLYV- Papaya lethal yellowing virus

PVY – *Potato virus Y*

RGMoV- *Ryegrass mottle virus*

RuCMoV – Rubus chlorotic mottle virus

RYMV- *Rice yellow mottle virus*

SBMV- *Southern bean mosaic virus*

SCMoV- *Subterranean clover mottle virus*

SCPMV – *Southern cowpea mosaic virus*

SeMV- *Sesbania mosaic virus*

SNMoV- *Solanum nodiflorum mottle virus*

SoMV- *Sowbane mosaic virus*

TMV- *Tobacco mosaic virus*

TuMV- *Turnip mosaic virus*

TRoV- *Turnip rosette virus*

VTMoV- *Velvet tobacco mottle virus*

WSMV- *Wheat streak mosaic virus*