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Characterisation and detection of viruses (*Cucumovirus, Potyvirus*) infecting vanilla in Réunion Island and Polynesian Islands

Karin Farreyrol

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor in Philosophy in Biological Sciences, The University of Auckland, 2005
Vanilla planifolia, Réunion Island

A. Cultivation plot under trees
B. Flowers in shade house
C. Vine bearing green pods
D. Cured beans

Pictures: K. Ferreyrol (A, B, C); N. Aikhoun (D)
Abstract

Natural vanilla (Vanilla planifolia, V. tahitensis) is economically important for a number of producing countries, but viral diseases are prejudicial to its successful cultivation. Techniques are required to detect viruses in vanilla plants in order to establish quarantine procedures and sources of virus-free planting material. This study contributed to the progress in managing viral diseases of vanilla by firstly identifying Cucumber mosaic virus (CMV, Cucumovirus) as causing severe distortion, stunting, sterility and sometimes death of vanilla plants. Vanilla CMV isolates from French Polynesia (Pacific Ocean) and Réunion Island (Indian Ocean) were classified in CMV subgroup IB, adding to the short list of subgroup IB isolates detected outside of Asia. Two isolates were putatively classified in subgroup IA, suggesting that CMV in vanilla is still evolving and/or that vanilla is infected from different sources. Subgroup II CMV from New Zealand was also experimentally infectious to V. planifolia, showing that vanilla crops should be protected from all potential sources of CMV inoculum. Existing serological and molecular detection tests were performant for the detection of CMV directly from vanilla tissue.

Secondly, this study provided the first coat protein sequence information for Vanilla mosaic virus (VanMV, Potyvirus). A Cook Islands isolate (VanMV-CI) and a French Polynesia isolate (VanMV-FP) had distinctly different coat proteins. The VanMV-FP CP N-terminus contained a stretch of amino-acid repeats (GTN) typical of natively unfolded proteins. This GTN stretch was located downstream of a DVG motif (which replaced the more common aphid transmission DAG motif), suggesting a role in improving aphid transmission, or regulating formation of the HC-virus complex.

CP core nucleotide sequence identities indicated VanMV-CI and VanMV-FP were strains of Dasheen mosaic virus (DsMV). In contrast, CP amino-acid sequence homologies between VanMV-CI and DsMV were intermediate between strains and species, and CP amino-acid homologies between VanMV-FP and DsMV were typical of distinct species. In addition, VanMV-CI and VanMV-FP had characteristic 3'NTR sequences and Nilb/CP cleavage sites, and only infected vanilla. Hence, it is proposed that VanMV-CI and VanMV-FP are considered new Potyvirus species and named Vanilla mosaic Cook Islands virus and Vanilla mosaic French Polynesia virus. Alternatively, the two isolates may be grouped under the name Dasheen mosaic virus-Vanilla (DsMV-V) and distinguished from Dasheen mosaic virus-Dasheen (DsMV-D).

Primers to VanMV-CI and VanMV-FP were designed and permitted RT-PCR detection of the viruses directly from vanilla tissue. VanMV-CI and VanMV-FP could be differentiated from DsMV and Watermelon mosaic virus (WMV-Tonga), and differentiated from each other by comparison of amplicon size. Long-term specific potyvirus diagnosis is however expected to be difficult due to potyviral variability in vanilla. Future research should concentrate on techniques such as
microarrays to permit simultaneous detection combined with specific identification of Potyvirus species. Such techniques would be beneficial to viral disease management in vanilla and many other crops.

Résumé

La vanille (Vanilla planifolia, V. tahitiensis) est une culture d’importance économique majeure pour quelques pays producteurs. Néanmoins, la présence de maladies, notamment virales, est susceptible d’affecter significativement la production. Des techniques de détection virale sont donc nécessaires à la mise en place de procédures de quarantaine et de certification phytosanitaire des boutures. En premier lieu, la présente étude a contribué à l’amélioration du contrôle des maladies virales dans les vanilleraies par l’identification du virus de la mosaïque du concombre (CMV, Cucumovirus) en tant que pathogène responsable de déformations sévères, nanisme, stérilité et parfois de la mort du vanillier. La détection fiable du CMV dans le vanillier est possible par le biais de tests ELISA et PCR existants. Des isolats de CMV du vanillier, récoltés en Polynésie Française et à l’Ile de la Réunion, ont été classés dans le sous-groupe IB des isolats de CMV, s’ajoutant à la courte liste des isolats IB hors-Asie. Deux isolats ont été provisoirement classés dans le sous-groupe IA, ce qui reflèterait une évolution en cours et/ou une origine multiple des souches de CMV infectant le vanillier. De plus, une souche de CMV appartenant au sous-groupe II a été mécaniquement transmise à des jeunes plants de V. planifolia. Ces résultats démontrent que les vanilleraies doivent être protégées de toute source potentielle de CMV.

En second lieu, cette étude a permis de déterminer pour la première fois la séquence du gène codant pour la capsides du virus de la mosaïque de la vanille (VanMV, Potyvirus). Il s’est avéré qu’un isolat des Iles Cook (VanMV-CI) et un isolat de Polynésie Française (VanMV-FP) ont des capsides différentes. La région N-terminale de la protéine de capsid du VanMV-FP possède une séquence de 76 acides aminés (GTN) typique de protéines dites ‘accordéon’. Cette séquence est située en aval d’un triplet DVG, qui remplace DAG – plus courant chez les potyvirus transmissibles par pucerons. Elle pourrait jouer un rôle d’amélioration de la transmission, ou de régulation lors de la formation du complexe HC-virus.

La comparaison des régions ‘core’ des protéines de capsid indique que le VanMV-CI et le VanMV-FP sont des souches du Dasheen mosaic virus (DsMV). Par contre, l’analyse des capsides entières place VanMV-CI à mi-chemin entre souche de DsMV et espèce potyvirale distincte, tandis que la capsides de VanMV-FP est plus clairement celle d’une espèce distincte. VanMV-CI et VanMV-FP se distinguent également du DsMV par leurs régions 3’NTR, leurs sites de clivage N1b/CP, et leur
gamme d’hôte restreinte au vanillier. Ainsi, il est proposé que VanMV-CI et VanMV-FP soient classés en tant que nouvelles espèces dans le genre *Potyvirus*, et nommés *Vanilla mosaic Cook Islands virus* et *Vanilla mosaic French Polynesia virus*. S’ils devaient être classés en tant que souches du DsMV, la distinction entre *Dasheen mosaic virus-Vanilla* (DsMV-V) et *Dasheen mosaic virus-Dasheen* (DsMV-D) devrait être considérée.

En dernier lieu, des amorces ont été sélectionnées pour la détection en RT-PCR du VanMV-CI et du VanMV-FP. Elles ont permis l’amplification d’un fragment du gène de capsid directement à partir de matériel végétal, ainsi que la différenciation des deux virus par la taille des produits d’amplification. Les amorces ont également permis de distinguer le VanMV du DsMV et du *Watermelon mosaic virus* (WMV-Tonga). Cependant, la variabilité des souches virales est susceptible d’entraîner à long terme des difficultés pour le diagnostic spécifique des potyvirus. Les futurs travaux de recherche devraient se concentrer sur la mise au point de techniques de type microarray, qui permettraient une détection à la fois spécifique et simultanée de toutes les espèces de *Potyvirus*. De telles techniques contribueraient à améliorer la protection phytosanitaire des vanilleraies et de nombreuses autres cultures.
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I want to say to all potential plant virology Ph.D. students at The University of Auckland that Dr Mike Pearson is a great supervisor! Thank you Mike for your knowledge and your constant support. I consider myself very lucky to have undertaken a Ph.D. under your supervision.

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Abbreviations

aa        Amino-acid
ACP-ELISA Antigen-coated plate - ELISA
bp        Base pairs
BSA       Bovin serum albumin
BYMV      Bean yellow mosaic virus
c.        Approximately (circa)
CalIMMV   Calanthe mild mosaic virus
cDNA      Complementary DNA
CerMV     Ceratobium mosaic virus
CI        Cylindrical inclusion
CIRAD     Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIYVV     Clover yellow vein virus
CMV       Cucumber mosaic virus
CP        Coat protein
cv.       Cultivar
CymMV     Cymbidium mosaic virus
DAS-ELISA Double antibody sandwich-ELISA
DenMV     Dendrobium mosaic virus
DiVY      Diurus virus Y
dNTP      Deoxyribonucleoside triphosphate
dsDNA     Double stranded DNA
DsMV      Dasheen mosaic virus
dsRNA     Double stranded RNA
ELISA     Enzyme-linked immunosorbent assay
EM        Electron microscopy
FDGDEC    Fédération Départementale des Groupements de Défense contre les Ennemis des Cultures
FDGDON    Fédération Départementale des Groupements de Défense contre les Organismes Nuisibles
FP        French Polynesia
i.e.      That is, that is to say, in other words (id est)
IgG       Immunoglobulin G
INRA      Institut National de la Recherche Agronomique
LB broth  Luria-Bertani broth
MAb       Monoclonal antibody
ML        Maximum likelihood
M-MLV     Moloney Murine Leukemia Virus
MP        Maximum parsimony
NI        Nuclear inclusion
NJ        Neighbour-joining
nt        Nucleotides
NTR       Non-translated region
ORF       Open reading frame
ORSV      Odontoglossum ring spot virus
PCR       Polymerase chain reaction
PPV       Plum pox virus
PSV       Peanut stunt virus
PTA       Potassium phosphotungstate
PtVY      Pterostylis virus Y
PVP       Polyvinyl pyrrolidone
RDP       Recombination Detection Program
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<td>RdRp</td>
<td>RNA-dependent RNA polymerase</td>
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<td>RhoVY</td>
<td>Rhopalanthe virus Y</td>
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<td>RT</td>
<td>Reverse Transcription</td>
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<td>RUN</td>
<td>Reunion Island</td>
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<td>SarVY</td>
<td>Sarcochilus virus Y</td>
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<td>SCMV</td>
<td>Sugarcane mosaic virus</td>
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<tr>
<td>sp.</td>
<td>Species (singular)</td>
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<tr>
<td>spp.</td>
<td>Species (plural)</td>
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<td>ssRNA</td>
<td>Single stranded RNA</td>
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<tr>
<td>TAS-ELISA</td>
<td>Triple Antibody sandwich-ELISA</td>
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<tr>
<td>TM</td>
<td>Melting (annealing) temperature</td>
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<td>TuMV</td>
<td>Turnip mosaic virus</td>
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<tr>
<td>VanMV</td>
<td>Vanilla Mosaic Virus</td>
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<tr>
<td>VPg</td>
<td>Viral protein genome-linked</td>
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<td>WMV</td>
<td>Watermelon mosaic virus</td>
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