Comparisons of genomic and pathological features among barley and grapevine infecting-isolates of Arabis mosaic virus

Aurélie Marmonier, Emmanuelle Vigne, Veronique Komar, Gerard Demangeat, Sébastien Besse, Paul Gugerli, Olivier O. Lemaire

▶ **To cite this version:**

Aurélie Marmonier, Emmanuelle Vigne, Veronique Komar, Gerard Demangeat, Sébastien Besse, et al.. Comparisons of genomic and pathological features among barley and grapevine infecting-isolates of Arabis mosaic virus. 16. Congrès du Conseil international pour l'étude des maladies virales et de type viral de la vigne ; 16. Meeting of the International Council for the Study of Virus and virus-like diseases of the Grapevine, Aug 2009, DIJON, France. hal-02754912

HAL Id: hal-02754912
https://hal.inrae.fr/hal-02754912v1

Submitted on 3 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

COMPARISONS OF GENOMIC AND PATHOLOGICAL FEATURES AMONG BARLEY AND GRAPEVINE INFECTING-ISOLATES OF ARABIS MOSAIC VIRUS

Aurélie MARMONIER [1 *], Emmanuelle VIGNE [1], Véronique KOMAR [1], Gérard DEMANGEAT [1], Sébastien BESSE [2], Paul GUGERLI [2], Olivier LEMAIRE [1]

[1] Institut National de la Recherche Agronomique, Unité Mixte de Recherche Santé de la Vigne et Qualité du Vin, Laboratoire de Virologie et Vection, 28 rue de Herrlisheim, 68021 COLMAR, France

[2] Agroscope Changins-Wädenswil Research Station ACW, Virology - Bacteriology, 1260, NYON 1, Switzerland

*Corresp. author: Aurélie Marmonier, Phone +33 3 89 22 49 69, Fax +33 3 89 22 49 33, Email: aurelie.marmonier@colmar.inra.fr

Summary

A new virus disease, named barley yellowing, has been identified in the 90's in Switzerland on winter barley. This disease has been associated to a strain of *Arabis mosaic virus*. In this study, virulence properties, host range, genetic composition and organization, sequences were determined and compared to known ArMV isolates.

INTRODUCTION

In the 1990's, striking yellows has been observed on the winter barley varieties Express and Mannitou, in five locations in the Swiss cantons of Fribourg and Bern (Gugerli *et al*., 1996). This disease, propagated by ectoparasitic nematodes, differs from the aphid-transmissible barley yellow dwarf disease, widespread in Switzerland, by the absence of mortality in the cereal crops. Biological, serological analyzes and partial nucleotide sequences indicate that a nepovirus, *Arabis mosaic virus-barley strain* (ArMV-ba), is the etiological agent.

ArMV belongs to the genus *Nepovirus* within the family *Comoviridae*. The genome of ArMV consists of two single-stranded positive-sense RNAs (ssRNA), RNA1 and RNA2 displaying a covalently attached small viral genome-linked protein (VPg) at their 5' end and a poly(A) stretch at their 3' end. Each genomic RNA encodes for a polyprotein from which functional proteins are released by proteolytic processing. A third component of variable length, characterized as the satellite RNA3, can be observed in some cases (Mayo & Robinson, 1996). ArMV, which is specifically transmitted by the ectoparasitic nematode *Xiphinema diversicaudatum,* has a wide natural host range including a number of economically important crop plants: grapevine, raspberry, strawberry, cucumber, sugar beet, lettuce, apple, cherry, rose, hop, petunia, narcissus, lilac, privet, etc. (Murant *et al.,* 1990).

ArMV-ba is the only ArMV strain known to naturally infect a graminaceous host. It causes more severe symptoms on *Chenopodium* and *Nicotiana spp* than most of the ArMV isolates from grapevine. The aim of our study was to collect data about the genome organization and pathological features of this nepovirus-infecting Gramineae. To address this issue, viral RNAs were extracted, cDNA were synthesized, amplified by PCR, subsequently cloned and sequenced. Biological assays were performed through grapevine natural inoculation by *in vitro* heterologous grafting and nematodes transmission.

MATERIAL AND METHODS

ArMV-ba was propagated on *Chenopodium quinoa* by mechanical inoculation. Viral RNA extractions were performed from purified virus particles and analyzed on denaturing formaldehyde gel.

To determine the genetic organization of ArMV-ba, total RNA was extracted from infected leaves of *C. quinoa* using RNeasy Plant Mini Kit (Qiagen, GmbH, Hilden, Germany). Viral RNA1 and -2 were characterized by RT-PCR with random, or specific and degenerate primers designed from available ArMV sequences. The 5' and 3' ends of the RNAs were determined using 5'/3' RACE kit (Roche Diagnostics, Germany). DNA products were cloned into pGEM-T plasmid (Promega Corporation, USA). Nucleotide sequences obtained from ABI 373 sequencing device, were then analyzed using the Vector NTI (Invitrogen, USA) bioinformatics software package. The program SiScan was used to confirm suspected recombination events (Gibbs *et al.,* 2000).

In vitro heterologous grafting was done between scions from healthy grapevines cuttings of *V. berlandieri* x *V. riparia* cvs Kober 5BB and rootstocks from *C. quinoa* previously mechanically inoculated with ArMV-ba as described by Belin *et al.,* 2001. After 3 weeks of contact, grapevines were separated from *C. quinoa* stems. After 6 weeks of *in vitro* culture, grapevine plants were acclimatized to greenhouse conditions. The presence of ArMV in these grapevines was assessed by DAS-ELISA with specific anti-ArMV γ globulins and by Immuno-Capture-RT-PCR (IC-RT-PCR).

The nematode transmission assay relies on a two-steps approach. First, aviruliferous *X. diversicaudatum* feeding on roots of ArMV-ba infected *C. quinoa* plants, were allowed for an acquisition access period of 6 weeks. Then, infected *C. quinoa* plants were removed and replaced by healthy bait grapevine for virus inoculation. At the end of the inoculation access period (6 weeks), the presence of virus was monitored in the bait grapevine roots by DAS-ELISA and IC-RT-PCR.

RESULTS AND DISCUSSION

Viral ssRNAs analyzed by denaturing gel electrophoresis, exhibited three distinct bands. Lengths of genomic RNA1 (7.4 kb) and -2 (3.8 kb) are in accordance with those of other ArMV isolates (Dupuis *et al*., 2008,

Imura *et al*., 2008, Loudes *et al.*, 1990, Vigne *et al.,* 2008, Wetzel *et al*., 2001; 2004). The smallest band of about 0.3 kb could correspond to the small circular satellite RNA3 already described for hop–infecting ArMV isolate (Kaper *et al*., 1988), thus differing from most ArMV satellites of c.a. 1.1 kb long (Liu *et al*., 1990; Wetzel *et al*., 2005). The sequence of the ArMV-ba RNA3 satellite exhibits a length of 301 nucleotides (nt) and shows 81,1 % identity with RNA3 of ArMV-*hop strain*, which is 300 nt long.

To determine complete sequences of RNA1 and -2, several overlapping DNA fragments were produced by RT-PCR. Several clones were sequenced from each cDNA fragment. Some of them showed nt identities around 90%, suggesting a strong complexity for genome organization of ArMV-ba. Indeed, the ArMV-ba genome harbors two RNA2 and three RNA1 molecules genetically distincts. Even if a doublet of RNA2 has been already described for the ArMV-S isolate (Loudes *et al.*, 1990), this is the first molecular characterization of a nepovirus encompassing multi RNA1 molecules.

The complete nt sequences of the two RNA2 molecules were 3811 and 3812 long. Both RNA2 sequences shared 93% identity. Each RNA2 contains a single open reading frame (ORF) encoding for a polyprotein P2 of a unique size of 1119 amino acids fitting with others P2 sizes encoded by the RNA2 of the ArMV isolates described by Dupuis *et al*.(2008), Imura *et al*. (2008), Loudes *et al.* (1990), Vigne *et al.* (2008) and Wetzel *et al*. (2001).

85 % of the full-length of the three RNA1 molecules was determined. The nt sequence comparisons pointed out variable regions (with 87% nt identity) and also regions displaying high level of identities (up to 98%).

Interestingly, two intra-specific recombination events were shown within the ArMV-ba RNAs: one that corresponds to a cross-over site within the RNA1-encoding putative helicase gene and another one within the RNA2-encoding coat protein gene. Recombination is a natural mechanism involved in genetic drift of plant viruses. It has been already described for RNA2 of *Grapevine fanleaf virus* (GFLV), a closely-related nepovirus also responsible for fanleaf disease of grapevine (Vigne *et al.,* 2004), but not yet for ArMV isolates. Our preliminary results indicate, for the first time, that recombination events could occur on RNA1 and -2 molecules of ArMV.

Complete sequence analyzes and phylogenetic relationships will be presented and discussed regardless of the genetic evolution and host adaptation of ArMV. A keypoint question to address, will be the ability of ArMV-ba isolate to infect grapevine, while keeping in mind its original genetic organization. Attempts to inoculate grapevine by *in vitro* heterologous grafting approach and by *X. diversicaudatum* transmission experiments will be also presented.

LITERATURE

BELIN, C., SCHMITT, C., DEMANGEAT, G., KOMAR, V., PINCK, L. & FUCHS, M. 2001. Involvement of RNA2-Encoded proteins in the specific transmission of *Grapevine fanleaf virus* by its nematode vector *Xiphinema index. Virology* 291, 161-171.

DUPUIS, L., COBANOV, P., BASSLER, A., KRCZAL, G. & WETZEL, T. 2008. Complete genome sequence of a virulent isolate of *Arabis mosaic virus* from privet (*Ligustrum vulgare*). *Archives of Virology* 153,1611-1613.

GIBBS, M.J., ARMSTRONG, J.S. & GIBBS, A.J. 2000. Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. *Bioinformatics* 16, 573-582.

GUGERLI, P. & RAMEL, M.E. 1996. Jaunisse de l'orge : une nouvelle virose associée à une souche du virus de la mosaique de l'arabette (ArMV), transmise dans le sol par le nématode *Xiphinema diversicaudatum* (Micoletzky) Thorne. *Revue suisse Agricole* 28 (6), 329-335.

IMURA, Y., OKA, H., KIMATA, K., NASU, M., NAKAHAMA, K. & MAEDA, T. 2008. Comparisons of complete RNA-2 sequences, pathological and serological features among three Japanese isolates of *Arabis mosaic virus*. *Virus Genes* 37, 333-341.

KAPER, J.M., TOUSIGNANT, M.E. & STEGER, G. 1988. Nucleotide sequence predicts circularity and self-cleavage of 300-ribonucleotide satellite of Arabis mosaic virus. *Biochemical and Biophysical Research Communications* 154 (1), 318-325.

LIU, Y.Y., HELLEN, C.U.T., COOPER, J.I., BERTIOLI, D.J., COATES, D. & BAUER, G. 1990. The nucleotide sequence of a satellite RNA associated with arabis mosaic nepovirus. *Journal of General Virology* 71, 1259-126.

LOUDES, A.M., RITZENTHALER, C., PINCK, M., REINBOLD, J., WALTER, B. & PINCK, L. 1990. The 119 kDa and 124 kDa polyproteins of arabis mosaic nepovirus (isolate S) are encoded by two distinct RNA2 species. *Journal of General Virology* 76, 899-906.

MAYO, M.A. & ROBINSON, D.J. 1996. Nepoviruses: Molecular biology and replication. In: Harrison B.D., Murant A.F., (Eds). *The Plant Viruses, vol.5: Polyedral Virions and Bipartite RNA Genomes* pp. 139-185. Plenum, New York, NY, USA.

MURANT, A.F., JONES, A.T., MARTELLI, G.P. & STACE-SMITH, R. 1990. Nepoviruses: general properties, diseases and virus identification. In: Harrison B.D., Murant A.F., (Eds). *The Plant Viruses, vol.5: Polyedral Virions and Bipartite RNA Genomes* pp. 99-138. Plenum Press, New York, NY, USA.

VIGNE, E., BERGDOLL, M., GUYADER, S. & FUCHS, M. 2004. Population structure and genetic variability within isolates of *Grapevine fanleaf virus* from a naturally infected vineyard in France: evidence for mixed infection and recombination. *Journal of General Virology* 85, 2435-2445.

VIGNE, E., MARMONIER, A. & FUCHS, M. 2008. Multiple interspecies recombination events within RNA2 of *Grapevine fanleaf virus* and *Arabis mosaic virus*. *Archives of Virology* 153, 1771-1776.

WETZEL, T., MEUNIER,,, JAEGER, U., REUSTLE, G.M. & KRCZAL, G. 2001. Complete nucleotide sequences of the RNAS 2 of German isolates of Grapevine fanleaf and Arabis mosaic nepoviruses. *Virus Research* 75, 139-145.

WETZEL, T., BECK, A., WEGENER, U. & KRCZAL, G. 2004. Complete nucleotide sequence of the RNA1 of a grapevine isolate of *Arabis mosaic virus*. *Archives of Virology* 149, 989-995.

WETZEL, T., BASSLER, A., AMREN, M.A.W. & KRCZAL, G. 2005. A RT/PCR-partial restriction enzymatic mapping (PREM) method for the molecular characterisation of the large satellite RNAs of *Arabis mosaic virus* isolates. *Journal of Virological Methods* 132, 97-103.

ACKNOWLEDGEMENTS

We are grateful to the Sequencing facility of the Institut de Biologie Moléculaire des Plantes, Centre National de la Recherche Scientifique, Strasbourg, France.