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First description of *Grapevine leafroll-associated virus 5* in Argentina and partial genome sequence

Sebastián Gómez Talquenca · Claudio Muñoz · Oscar Grau · Olga Gracia

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Abstract An accession of *Vitis vinifera* cv. Red Globe from Argentina, was found to be infected with *Grapevine leafroll-associated virus-5* by ELISA. It was partially sequenced, and three ORFs, corresponding to HSP70h, HSP90h, and CP, were found. This isolate shares a high aminoacid identity with the previously reported sequence of the virus, and identities between 80% and 90% with previously reported GLRaV-9 and GLRaV-4 isolates. The analysis of the sequence supports the clustering together with GLRaV-4 and GLRV-9 inside the *Ampelovirus* genus.

Keywords GLRaV-5 · *Ampelovirus* · Grapevine

Leafroll is one of the most deleterious viral diseases of grapevines across the world, causing severe losses owing to poor yields and quality. Nine viruses have been reported associated with the disease, all belonging to the *Closteroviridae* family. Eight of them are definitive or tentative members of the *Ampelovirus* genus whereas one belongs to the *Closterovirus* genus [6].

The nucleotide sequence data reported in this article has been submitted to the GenBank nucleotide sequence database and has been assigned the accession numbers EU815935.
The authors S. Gómez Talquenca and C. Muñoz have contributed equally to this work.

S. Gómez Talquenca (✉) · C. Muñoz · O. Gracia
Plant Virology Laboratory, EEA Mendoza INTA, San Martín 3853, (5507) Luján de Cuyo, Mendoza, Argentina
e-mail: gtalquenca@mendoza.inta.gov.ar

O. Grau
CIC Bs. As.-IBBM-CCT-CONICET-Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Buenos Aires, Argentina

Grapevine leafroll-associated virus-5 (GLRaV-5) is one of the definitive species of the *Ampelovirus* genus and would share the same phylogenetic cluster with GLRaV-4, 6, 9, and *Pineapple mealybug wilt-associated virus* (PMWaV)-1 and 4 [5, 7]. GLRaV-5 has been partially sequenced [4] and reported in several grape growing countries. Sequence analysis revealed the presence of a closterovirus HSP70, a 52.6 kDa protein similar to but shorter than HSP90h found in other ampeloviruses, and putative CP (29.3 kDa) and divergent CP (23.1 kDa) coding sequences, in the same order as in other ampeloviruses.

In Argentina, leafroll is one of the most important viral diseases of grapevine. However, only GLRaV-1 and GLRaV-3, which belong to the *Ampelovirus* genus, and GLRaV-2 which belongs to the *Closterovirus* genus, had been found [3].

In the course of a survey for *Closteroviridae* family members [3], a Red Globe (RG) grapevine obtained from a nursery located in the east of Mendoza province was grown in the Mendoza Research Station of INTA (located at 33°00′S and 68°52′W). Double-stranded RNA (dsRNA) was extracted from cortical scrapings and amplified to obtain a fragment of HSP70h. The restriction size of the fragments generated with HinfI did not correspond to GLRaV-1, 2, or 3 (data not shown). The sample was then analyzed by DAS-ELISA for GLRaV-1, GLRaV-2, GLRaV-3 (Bioreba AG, Switzerland), GLRaV-5 (Sediag, France), and GLRaV-7 (Agritest, Italy), and found to be co-infected with GLRaV-2 and GLRaV-5.

DsRNA was extracted from mature canes according to the technique described by Valverde et al. [9]. The dsRNA was random primed and reverse transcribed. cDNA was amplified by PCR to generate specific fragments of the HSP70h (using the primers described in [3] for the *Closteroviridae* family members) and GLRaV-5 CP [4].The

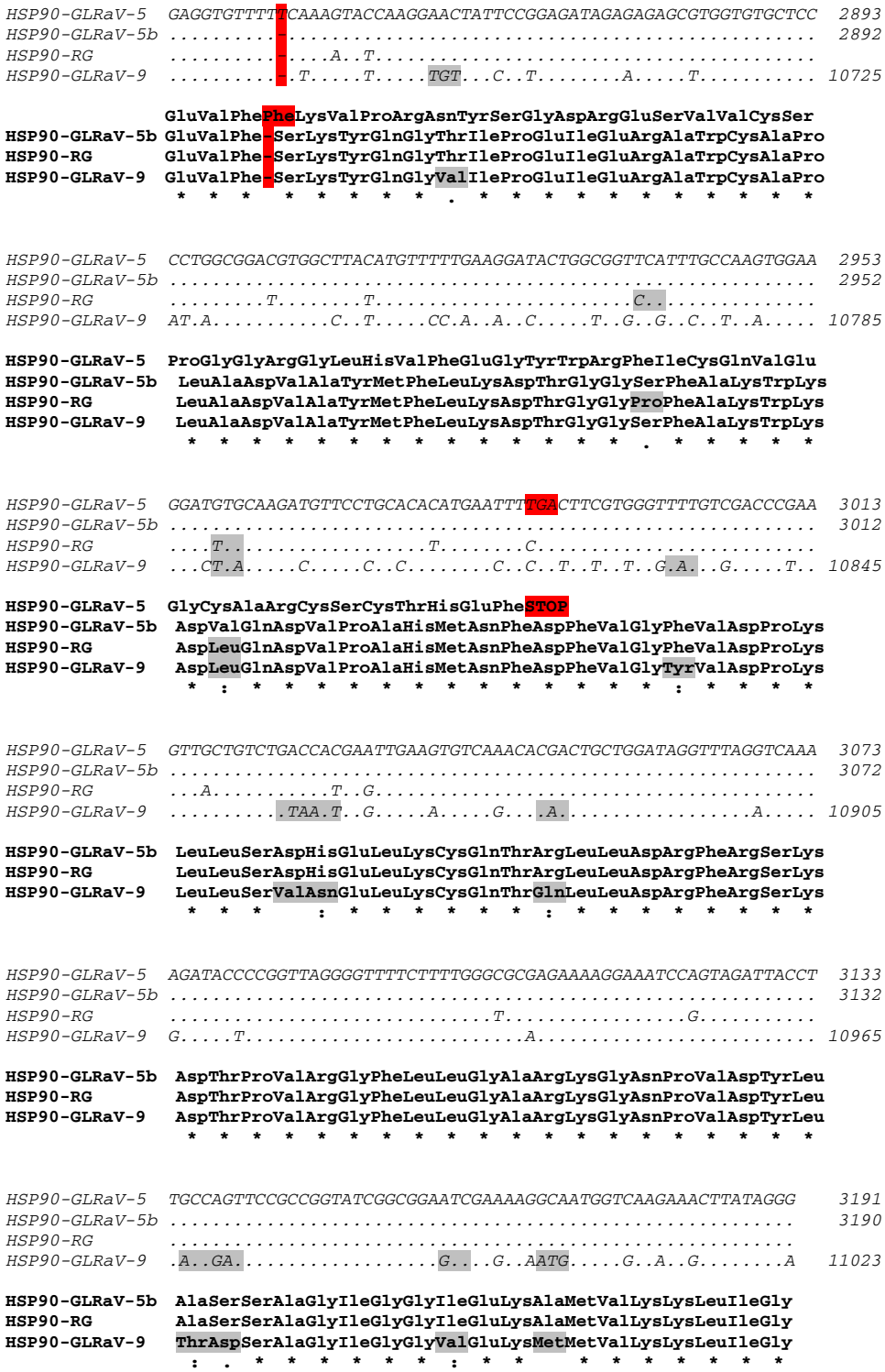

Fig. 1 Multiple alignment of the 3′ end of the nucleotide sequence (*italics*) and deduced aminoacid sequence (*bold*) of HSP90h ORF of GLRaV-5 (AF233934), the proposed correction of the sequence (GLRaV-5b), the RG strain (EU815935), and GLRaV-9 (AY297819). Shaded in *red* is the single nucleotide deletion and its effect. Shaded in *gray* is the non-synonymous nucleotide substitutions and derived aminoacid change

PCR products showed the expected size. A 579 bp internal fragment in the HSP90h ORF was obtained by the method of Fazelli et al. [2]. The PCR products were cloned using the pGEM-T Easy Vector System (Promega) and sequenced using an ABI 3130 Genetic analyzer. Primer3! Program [8] was used to design primers able to amplify the

sequences between the obtained clones. These PCR products were cloned and sequenced as described. The sequences corresponded in size with the published data for HSP70h and CP GLRaV-5. The fragment corresponding the HSP90h showed a single nucleotide deletion in the T^{2844} of the reference sequence. This single nucleotide deletion produces a translation product of 68 aminoacid larger than the reference sequence (Fig. 1). The sequence files were assembled using the Contig Express software (Vector NTI Suite 8, Informax, Inc.). The analysis of 4,100 nucleotides sequenced showed three ORFs, corresponding to HSP70h, HSP90h, and CP, in the same order as in the GLRaV-5 type strain. A phylogenetic analysis was done over a multiple sequence alignment (ClustalX) by bootstrapping and parsimony using the PHYLIP package with all sequences, and the corresponding genomic sequence of GLRaV-5, GLRaV-9, and PMWaV-1 and GLRaV-3 as outgroup. A distance matrix for each protein was calculated with the Protdist program (PHYLIP package), obtaining identities higher than 90% for isolates of the same species (GLRaV-5 or GLRaV-4), between 80% and 90% for GLRaV-4, 5, and 9, and lower identities for PMWaV-1 and GLRaV-3.

The deletion observed in the HSP90h ORF, together with the analysis of GLRaV-6 (Gómez Talquenca, unpublished results) and GLRaV-9 [1] sequences, suggest an error in the sequence reported for GLRaV-5 type strain, because a repetitive "T" sequence could be misread by the sequencer. In the multiple alignment of Fig. 1, the deletion of the T^{2844} of the AF233934 sequence modifies the ORF, and the new translated protein has the same length of HSP90 as from our isolate and GLRaV-9. The level of identity between the HSP90 protein from the RG isolate, and the corrected GLRaV-5 sequence is 96% (against 80% before the correction). In addition, the identity of the N-terminal sequences of GLRaV-9, our RG isolate and the corrected sequence of GLRaV-5, is higher than 90%, and the size of the products is the same (Fig. 1). These sequences display a variable aminoacid identity level with the reference strain (92% for the HSP70h, 96% for the corrected HSP90h, and 96% for the CP), and high homology against GLRaV-4 and GLRaV-9, confirming the previously proposed clustering of GLRaV-4, 5, and 9 [5]. These results also support the clustering suggested by Melzer et al. [7], although PMWaV-1 is distantly related to the grapevine *Ampelovirus*.

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References

1. R. Alkowni, A. Rowhani, S. Daubert, D. Golino, J. Plant Pathol. **86**, 123–133 (2004)
2. C.F. Fazeli, N. Habili, M.A. Rezaian, J. Virol. Methods **70**, 201–211 (1998). doi:10.1016/S0166-0934(97)00193-6
3. G.S. Gómez Talquenca, O. Gracia, S. García Lampasona, O. Grau, A survey for Closteroviridae family members in Argentinean vineyards, in *Proceedings of the 14th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine* (Locorotondo, Italy, 2003), pp. 43–44
4. X. Good, J. Monis, Phytopathology **91**, 274–281 (2001). doi: 10.1094/PHYTO.2001.91.3.274
5. G.P. Martelli, Grapevine virology highlights 2004–2005, in *15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine—Extended Abstracts* (Stellenbosch, Sudáfrica, 2006), pp. 3–18
6. G.P. Martelli, A.A. Agranovskaii, M. Bar-Joseph, D. Boscia, T. Candresse, R.H.A. Coutts, V. Dolja, B·W. Falk, D. Gonsalves, J.S. Hu, W. Jelkmann, A.V. Karasev, A. Minafra, S. Namba, H.J. Vetten, C.G. Wisler, N. Yoshikawa, in *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, ed. by C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A. Ball (Elsevier Academic Press, 2005)
7. M.J. Melzer, D.M. Sether, A.V. Karasev, W. Borth, J.S. Hu, Arch. Virol. **153**, 707–714 (2008). doi:10.1007/s00705-008-0051-8
8. S. Rozen, H. Skaletsky, in *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, ed. by S. Krawetz, S. Misener (Humana Press, 2000)
9. R.A. Valverde, S.T. Nameth, R.L. Jordan, Plant Dis. **74**, 255–258 (1990). doi:10.1094/PD-74-0151