Identification of a new polymorphism in the MP/CP region of Spanish isolates of Grapevine Pinot gris virus

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Grapevine Pinot gris virus (GPGV) genome consists of one molecule of positive single stranded RNA, ranging from 7223 to 7275 nucleotides excluding the 3' polyA tail. The genome contains three overlapping open reading frames (ORFs) and two untranslated regions (UTRs) located at both the 5' and the 3' ends. ORF1 encodes a methyltransferase, a helicase and the RNA dependent RNA polymerase (RdRp), ORF2 encodes the movement protein (MP) and ORF3 encodes the coat protein (CP). Several studies have reported the existence of high variability in GPGV genomes. Among this genomic diversity, a polymorphism involving the MP stop codon that produces a six amino acids shorter protein has been described. Although this polymorphism has been related to the symptoms observed in GPGV infected grapevines, a association between this genetic variant and the manifestation of symptoms remains unclear. Phylogenetic studies based on the GPGV MP/CP genomic region have allowed to cluster GPGV isolates in different clades, being some of them generally associated to the presence or absence of symptoms. In the field, the GPGV-infected grapevines are often simultaneously affected by other viruses, making difficult to attribute a specific etiology to the GPGV infection. In addition, other factors such as viral titer have been reported to play an important role in the development of the disease. In the current study a new polymorphism, not previously reported, has been identified in the MP/CP region of Spanish isolates. This polymorphism involves the presence of a translational stop signal one codon downstream from the polymorphism reported. The presence of this single nucleotide polymorphism would determine the synthesis of a five amino acids shorter MP.

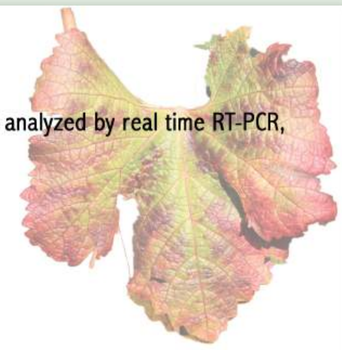
MATERIALS AND METHODS

Plant material and RNA isolation

178 grapevine samples from a random survey on a Spanish grapevine growing area (D.O. Utiel-Requena) were analyzed.

Total RNA from leaf tissue was extracted using the Plant/Fungi Total RNA Purification Kit (Norgen Biotek Corporation, Thorold, ON, Canada) following the manufacturer instructions.

Detection of other grapevine viruses



All the grapevine samples that tested positive for GPGV were analyzed by real time RT-PCR, for the presence of the following viruses:

-*Grapevine leafroll-associated virus* 1 (GLRaV-1)
.*Grapevine leaf- roll-associated virus* 2 (GLRaV-2)
-*Grapevine leafroll-associated virus* 3 (GLRaV-3)
-*Grapevine fleck virus* (GFkV)
-*Grapevine fanlef virus* (GFLV)
-*Arabis mosaic virus* (ArMV)

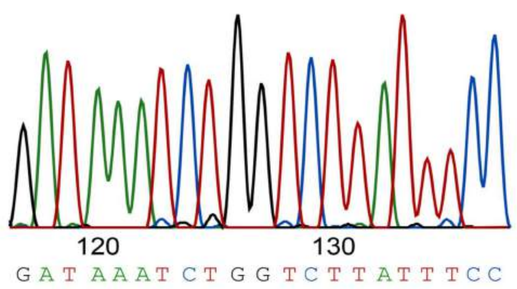
MP polymorphism of GPGV Spanish isolates

20 GPGV positive samples were used to amplify the 228 bp fragment of the MP/CP genes using AgPath-ID One-step RT-PCR kit (Ambion Inc., Austin, TX, USA) by conventional RT-PCR

0.9 µM of each of the primers GPGV-RT-F and GPGV-RT-R
3 µl of RNA.

RT-PCR protocol consisted of one step of 4º C for 30 min and 95ºC for 10 min followed by 45 cycles of amplification (95º C for 30 s, 50˚C for 30 s and 60 ˚C for 1 min).

Sanger sequences were aligned using Mega 6 software.



Phylogenetic analysis of GPGV Spanish isolates

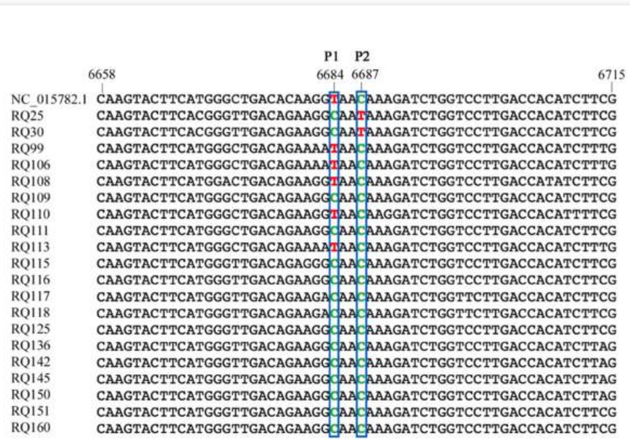
A 548 bp fragment of the GPGV MP/CP gene was amplified from 12 GPGV Spanish isolates by conventional RT-PCR using the primers Det-F and Det-R.

Maximum likelihood tree was obtained using the best nucleotide substitution model (Tamura 3) using Mega 6 software.

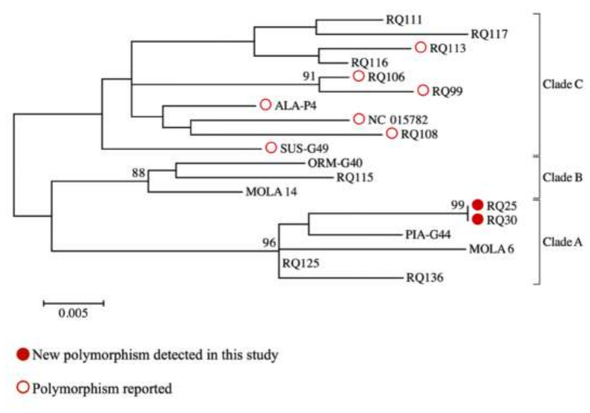
12 sequences (RQ) from spanish isolates were included in the phylogenetic analysis (GenBank accession numbers MH019203, MH019204, MH019205, MH019206, MH019207, MH019208, MH019209, MH019210, MH019211, MH019212, MH019213 and MH019214).

8 reference sequences were included in the alignment:
MOLA 14, MOLA 6, ALA-P4, ORM-G40, SUS-G49, PIA-G44, SK30 and SK01

RESULTS



Sequence alignment of the MP/CP region targeted by the real-time RT-PCR assay of 20 GPGV Spanish isolates using GPGV IT isolate (NC_015782.1). Two polymorphisms producing a shorter MP: P1 (previously reported) at position 6,684 and P2 (new polymorphism identified in this work) at position 6,687 are indicated.



Spanish isolates (RQ) did not cluster in any particular clade and are distributed along all the three clades.

Two isolates (RQ30 and RQ25) showing the new MP/CP polymorphism did not group in the same clade where isolates with the polymorphism previously reported are located.

Symptomatology and presence of other grapevine viruses in GPGV positive samples collected from D.O. Utiel-Requena.

Sample	Viral titer	Symptoms	Other viruses[b]
RQ25	1.7 x 10^5	chlorotic mottling and leaf deformation	GFLV, GLRaV-3
RQ30	3.9x 10^5	chlorotic mottling and leaf deformation	GFkV, GFLV, GLRaV-3
RQ99	1.3 x10^6	chlorotic mottling	GFLV, GFkV, GLRaV-2
RQ106	4.5 x10^5	symptomless	GLRaV-2
RQ108	4.4 x 10^5	chlorotic mottling	GFLV
RQ109	4.4 x 10^5	chlorotic mottling	GFLV
RQ110	4.4 x 10^5	chlorotic mottling	GFLV
RQ111	5.2 x 10^5	chlorotic mottling	GFLV
RQ113	1.8 x 10^6	chlorotic mottling	GFLV
RQ115	2.0 x 10^5	chlorotic mottling	GFLV
RQ116	7.5 x 10^4	chlorotic mottling	-
RQ117	1.1 x 10^5	chlorotic mottling	-
RQ118	4.6 x 10^4	chlorotic mottling	-
RQ121	3.6 x 10^3	symptomless	GLRaV-3, GFkV
RQ124	7.8 x 10^3	symptomless	GFLV
RQ125	4.3 x 10^5	symptomless	GFLV, GLRaV-2
RQ136	2.6 x 10^5	chlorotic mottling	GFLV
RQ140	5.7 x 10^3	chlorotic mottling	GFLV
RQ142	3.2 x 10^3	symptomless	GLRaV-2, GLRaV-3
RQ143	1.6 x 10^3	chlorotic mottling	GLRaV-2, GLRaV-3
RQ145	4.2 x 10^3	chlorotic mottling	GFLV
RQ150	2.3 x 10^3	chlorotic mottling	GFLV
RQ151	2.6 x 10^3	chlorotic mottling	GFLV
RQ160	3.0 x 10^3	chlorotic mottling	GFLV

CONCLUSIONS

- A new polymorphism in the MP/CP region of the GPGV genome has been identified.

-The presence of this single nucleotide polymorphism would determine the synthesis of a five amino acids shorter MP

-Not clear association between the polymorphism identified in this work and the symptomatology of the plant, in opposition to a similar polymorphism previously described.

-The phylogenetical analysis of the GPGV Spanish isolates points to that this new polymorphism represents an independent evolutionary event.

Reference: Morán F, Olmos A, Lotos L, Predajňa L, Katis N, Glasa M, Maliogka V, Ruiz-García AB. A novel specific duplex real-time RT-PCR method for absolute quantitation of Grapevine Pinot gris virus in plant material and single mites. PLOS ONE. 2018;13(5):e0197237.