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Additional Information

1 **Polyvalent detection of members of the Potyvirus genus by molecular**
2 **hybridization using a ‘Genus-probe’**

3
4 Jesús A. Sánchez-Navarro^{1*}, Christopher N. Cooper² and Vicente Pallás¹

5
6 *Author for correspondence: jesanche@ibmcp.upv.es

7
8 ¹Department of Molecular and Evolutionary Plant Virology. Instituto de Biología
9 Molecular y Celular de Plantas (IBMCP) (UPV-CSIC). Universitat Politècnica de
10 Valencia, Ingeniero Fausto Elio s/n, 46022 Valencia, Spain.

11 ²Georgia Institute of Technology. School of Chemistry & Biochemistry, Atlanta, GA
12 30332, United States.

13
14 Running title: Detection of Potyvirus genus

15 Keywords: molecular hybridization, Polyprobe, dig-RNA probe, genus-probe, Potyvirus

16

1 **ABSTRACT**

2 The use of a unique riboprobe named 'polyprobe', carrying partial sequences of
3 different plant viruses or viroids fused in tandem, has permitted the polyvalent detection
4 of up to ten different pathogens by using a non-radioactive molecular hybridization
5 procedure. In the present analysis, we have developed a unique polyprobe with the
6 capacity to detect all members of the *Potyvirus* genus, which we have named 'genus-
7 probe' (GP). To do this, we have exploited the capacity of the molecular hybridization
8 assay to cross-hybridize with related sequences by reducing the hybridization
9 temperature. We observed that sequences showing a percentage similarity of 68% or
10 higher, could be detected with the same probe by hybridizing at 50-55°C, with a
11 detection limit of picograms of viral RNA comparable to the specific individual probes.
12 According to this, we developed several polyvalent polyprobes, containing 3, 5 or 7
13 different 500-nucleotide fragments of a conserved region of the NIb gene. The
14 polyprobe carrying 7 different conserved regions was able to detect all the 32
15 potyviruses assayed in the present work with no signal in the healthy tissue, indicating
16 the potential capacity of the polyprobe to detect all described, and probably
17 uncharacterized, potyviruses being then considered as a genus-probe. The use of this
18 technology in routine diagnosis not only for *Potyvirus* but also to other viral genera is
19 discussed.

20

21

1 INTRODUCTION

2 Plants are affected by a large number of viruses, which in many cases cause
3 significant economic losses, compromising the viability of agricultural industries.
4 Effective management of viral diseases requires an integrated approach addressed to
5 prevent or delay the progress of the infection. The use of healthy plants represents one
6 of the main measures to manage viral diseases by reducing the initial source of
7 inoculum. Accordingly, rapid and reliable routine virus testing procedures is a critical
8 step in their control. Traditional detection methods include bioassays with indicator
9 plants and serological methods such as the enzyme-linked immunosorbent assay
10 (ELISA), although the molecular detection techniques (RT-PCR, microarrays,
11 molecular hybridization, etc) have been incorporated in the routine diagnosis of plant
12 viruses (Jeong et al., 2014).

13 During recent years, plant virus detection procedures have been addressed to get
14 the simultaneous detection of the main pathogens affecting a crop, in order to save time,
15 labor and overall cost. Several strategies have been used for simultaneous detection of
16 plant viruses. For PCR-derived assays, two main approaches have been used according
17 to the use of a cocktail of primers targeting different pathogens (multiplex RT-PCR) or
18 a polyvalent primer pair (polyvalent PCR) that is able to drive the amplification of a
19 conserved region (James et al., 2006). However, the detection limit of the multiplex RT-
20 PCR technique appears to be affected when more than six different pathogens are
21 detected (Sanchez-Navarro et al., 2005) and the polyvalent primers are often
22 compromised by the lack of conserved regions. In the case of the genus *Potyvirus*, the
23 presence of a very conserved region in the Nib gene, has permitted the detection of
24 virus isolates from all major groups of the genus, including uncharacterized species
25 (Zhen et al, 2010). The incorporation of the DNA-microarray technology has
26 incremented significantly the simultaneous detection and identification of multiple plant
27 viruses and (or) virus subgroups (Bystricka et al., 2005; Barba and Hadidi, 2011).
28 However, the difficulty of adapting the array technology for routine screening of large
29 numbers of samples and the high costs associated with equipment required have
30 compromised its incorporation in routine plant virus analysis. The serological assay has
31 been also adjusted to polyvalent detection of 33 (Jordan and Hammond, 1991) or 14
32 (Liu et al., 2015) potyvirus species by using broad-spectrum monoclonal antibody
33 (MAb) that recognize a conserved core region of the potyvirus coat proteins. In the

1 latter case, the authors indicate that the MAb could detect many potyviruses in infected
2 plants, but also that different binding affinities were observed in some infected samples.

3 Multiple detection by using the non-isotopic molecular hybridization technique
4 was addressed first by a cocktail of the specific single probes in the hybridization
5 solution, allowing the polyvalent detection of the different plant viruses affecting
6 ornamental (Sánchez-Navarro et al., 1999), horticultural (Saldarelli et al., 1996;
7 Minutillo et al., 2012) and stone fruits crops (Saade et al., 2000); and second, by using a
8 unique riboprobe, called 'polyprobe', that contains partial nucleic acid sequences of
9 different viruses (Herranz et al., 2005; Aparicio et al., 2009; Peiro et al., 2012;) or
10 viroids (Cohen et al., 2006, Lin et al., 2011, Zhang et al., 2012) cloned in tandem. The
11 polyprobe has proved the simultaneous detection of six different viruses (Herranz *et al.*,
12 2005) or up to eight viroids (Cohen et al., 2006; Torchetti et al., 2012), eight viruses
13 plus two viroids (Peiro et al., 2012) or three different pathogens comprising of virus,
14 viroid and bacteria (Zamora-Macorra et al., 2015), with the same detection limit as the
15 single assay. Recently, an octamer of 32-nucleotide sequence derived from the central
16 conserved region of viroids in the genus Coleoviroid was used to develop a universal
17 probe allowing the detection at least of four coleus viroids (Jiang et al., 2013).

18 In the present work, we have explored the potential capacity of the polyprobe
19 technology to develop an universal probe with the property to detect all members of a
20 specific viral genus, in this case the genus *Potyvirus*. The cross hybridization observed
21 at 50-55°C between sequences sharing an identity of 68% or higher was used to design
22 several polyprobes, carrying in tandem different 500-nucleotide fragments of the
23 conserved N1b gene, with the capacity to cross hybridize with all potyvirus species
24 available in the data base. A polyprobe carrying 7 different conserved regions was able
25 to detect all the 32 potyviruses assayed in the present work, revealing the potential
26 capacity of this technology to be applied at the genus-probe level.

30 MATERIAL AND METHODS

31 Computing analysis

1 The phylogenetic analysis was performed with all potyviruses for which the
2 complete nucleotide sequence is available in the database. The phylogenetic analyses
3 were inferred in a multi-step process: in the first step, the sequences were aligned using
4 the CLUSTAL W program (Higgins et al., 1994) to generate, in a second step, the
5 neighbour-joining phylogenetic trees, using the JTT model, implemented in the
6 MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets
7 (Kumar et al., 2016). Third, the statistical reliability of the constructed trees was
8 assessed by the bootstrap method based on 10,000 pseudoreplicates.

9 The more conserved region observed in the potyvirus alignment, covering 500
10 nucleotides of the NIb gene (Supplementary Figure 1) was used to estimate the identity
11 value between all the analysed potyvirus sequences. To do this, we used the MatGAT
12 (Matrix Global Alignment Tool) application that generates similarity/identity matrices
13 for DNA sequences (Campanella et al., 2003).

14 To identify the equivalent conserved region in the rest of viruses assigned to the
15 family *Potyviridae*, the seven selected conserved regions from *Lettuce mosaic virus*
16 (LMV), *Watermelon mosaic virus* (WMV), *Potato virus Y* (PVY), *Pepper veinal mottle*
17 *virus* (PVMV), *Plum pox virus* (PPV), *Sweet potato feathery mottle virus* (SPFMV) and
18 *Tobacco etch virus* (TEV) were aligned with all species of the corresponding
19 Potyviridae family, for which the complete nucleotide sequence is available in the
20 database, using the CLUSTAL W program. The selected regions (Supplementary Figure
21 1) were also used to estimate the identity value with the MatGAT application.

22

23 **Plant materials**

24 The plant tissue was obtained from the Leibniz Institute DSMZ-German
25 Collection of Microorganism and Cell Cultures (<https://www.dsmz.de/home.html>) or
26 from the Mediterranean Agroforestral Institute at the Polytechnic University of Valencia
27 (<http://www.upv.es/iam/ingles/bienvenida.htm>). Total nucleic acid extraction was
28 performed using 0.1 g of lyophilized or fresh leaf tissue using the Silica capture
29 extraction protocol (MacKenzie et al., 1997) that renders total nucleic acids. The
30 extracted nucleic acids were stored at -80 °C until use. Alternatively, healthy and

1 infected tissue were homogenized with 5 volumes of cold extraction buffer (50mM
2 sodium citrate, 5mM EDTA, pH 8.5) and directly applied (1µl) onto nylon membranes
3 (Sanchez-Navarro et al., 1998).

4

5 **Synthesis of cDNA clones**

6 Reverse transcription and PCR reactions were carried out using specific primers
7 (Table 1), containing the 5' and 3' *XhoI* and *SalI* restriction sites, respectively. PCR
8 products were digested with both restriction enzymes and extracted from the agarose
9 gel. The purified PCR fragments were inserted in the pBluescript SK(+) plasmid,
10 previously digested with the *XhoI* enzyme and dephosphorylated. The incorporation of
11 the purified PCR fragment into the pSK+ plasmid in the right orientation allowed the
12 inactivation of the original *XhoI* site, present in the pSK+ plasmid, by the compatible
13 *SalI* site. This permits the use of the new 5' proximal *XhoI* for the synthesis of the
14 riboprobe or the incorporation of a new PCR fragment (Peiró et al. 2012). Using this
15 strategy, we introduced seven cDNA fragments in the pSK+ corresponding to the partial
16 sequences of the following viruses: *Lettuce mosaic virus* (LMV), *Watermelon mosaic*
17 *virus* (WMV), *Potato virus Y* (PVY), *Pepper veinal mottle virus* (PVMV), *Plum pox*
18 *virus* (PPV), *Sweet potato feathery mottle virus* (SPFMV) and *Tobacco etch virus*
19 (TEV). We generated three polyprobes that differed in the number of viral sequences
20 incorporated. Thus, GP3, GP5 and GP7 contained three (PPV-SPFMV-TEV), five
21 (PVY-PVMV-PPV-SPFMV-TEV) and seven (LMV-WMV-PVY-PVMV-PPV-
22 SPFMV-TEV) viral sequences, respectively (Fig. 2).

23

24 **Synthesis of the digoxigenin-labeled riboprobes and hybridization procedure**

25 For the synthesis of the riboprobes, 1 µg of the corresponding plasmid was
26 linearized with *XhoI* restriction enzyme, purified by phenol–chloroform extraction and
27 precipitated with ethanol. The linearized plasmid was used to synthesize the riboprobe
28 as described previously (Mas et al. 1993; Pallás et al. 1998). 1 µl of the total nucleic
29 acids preparations (undiluted or serially diluted in extraction buffer), were directly
30 applied onto positively charged nylon membranes (Roche Diagnostics GmbH,

1 Manheim, Germany), air dried and cross-linked by UV crosslinker ($700 \times 100 \mu\text{J}/\text{cm}^2$).
2 Prehybridizations and hybridizations with the riboprobes were conducted as described
3 previously, with the only difference of the temperature selected for the hybridization
4 (Pallás et al. 1998; Sánchez-Navarro et al. 1999). All riboprobes were used at the same
5 concentration in the hybridization solution (20 ng/ml). Chemiluminiscent detection
6 using CDP-start reagent as substrate was performed as recommended by the
7 manufacturer (Roche Diagnostics GmbH, Manheim, Germany). Films were exposed for
8 30 minutes.

9 **Estimation of the detection limit of the hybridization assays**

10 For the estimation of the detection limit of the single or the three polyprobes, the
11 seven DNA fragments cloned in the pSK+ were PCR amplified using the corresponding
12 antisense primer and the reverse primer. The resultant PCR fragment contains the
13 corresponding potyvirus clone plus the T3 promoter. The amplicons were purified from
14 agarose gel and then used directly for the synthesis of unlabelled transcripts
15 complementary to the corresponding dig-RNA probes. Known amounts of the free
16 transcripts were serially diluted (five-fold) in sterile water since previous results showed
17 similar detection limit (pg/ μl of viral RNA) for the no-radioactive molecular
18 hybridization procedure when the dilutions were performed in sterile water (Peiró et al.,
19 2012) or healthy tissue (Sanchez-Navarro et al., 1996; 1998). The dilutions were applied
20 directly onto nylon membranes and the dot-blot hybridization was conducted as
21 described previously.

22

23 **RESULTS**

24 **Design of the polyprobes and analysis of the cross-hybridization**

25 In order to get representative common sequences that would allow us to design a
26 potential polyprobe at the genus level we performed a phylogenetic analysis using all
27 potyvirus sequences from 94 species. The resultant phylogenetic tree grouped all
28 viruses into different clusters supported with significant bootstrap values (Figure 1). We
29 selected the following seven potyviruses that cover the full spectrum of disparate
30 sequences (viral names in gray boxes, Figure 1): *Lettuce mosaic virus* (LMV),

1 *Watermelon mosaic virus* (WMV), *Potato virus Y* (PVY), *Pepper veinal mottle virus*
2 (PVMV), *Plum pox virus* (PPV), *Sweet potato feathery mottle virus* (SPFMV) and
3 *Tobacco etch virus* (TEV). Sequence alignment of all the 94 potyvirus species revealed
4 a highly conserved region of 500 nt in the N1b gene (Supplementary Figure 1). The
5 corresponding regions of the seven selected potyvirus were cloned separately in a
6 bacterial plasmid to generate the individual probes but also in tandem to obtain three
7 different polyprobes carrying 3 (PPV, SPFMV, TEV), 5 (PVY, PVMV, PPV, SPFMV,
8 TEV) or 7 (LMV, WMV, PVY, PVMV, PPV, SPFMV, TEV) viral sequences (Figure
9 2A). MatGAT analysis of the 7 selected sequences revealed a percentage identity that
10 ranged between 64.8% and 72.5% (Figure 2B).

11 In the next step, we analyzed the capacity of each individual riboprobe to cross-
12 hybridize to the rest of the selected sequences. To do this, we synthesized
13 complementary transcripts of the 7 selected sequences that were serially diluted (1:5)
14 and applied on nylon membranes, together with total RNA extracted from several
15 healthy tissues (Figure 3). Replicas of the same membrane were hybridized first with
16 the PPV riboprobe at different hybridization temperatures, to evaluate the effect of such
17 parameter in the cross-hybridization (Figure 3A). We observed no cross-reaction when
18 the hybridization was performed at 68°C. However, the reduction of 8 degrees during
19 the hybridization assay (60°C) was sufficient to start to see cross-hybridization with the
20 TEV sequence at high concentration (200 pg/μl), meanwhile the reduction of another 5
21 (55°C) or 10 (50°C) degrees was sufficient to detect two (TEV, SPFMV) or all
22 sequences at different concentrations. In the case of the hybridization performed at 50°C
23 we also observed a weak signal in the negative controls (100ng of total RNA extracted
24 from healthy tissue). Accordingly, we evaluated the cross-hybridization of the
25 remaining riboprobes at 55°C (Figure 3C). In general, we observed that sequences with
26 an identity percentage below 65 % with the corresponding probe were not detected at
27 55°C but also that sequences with such percentage higher than 67% were all detected,
28 except the PPV probe that rendered negative results with LMV, which shared 69.7%
29 identity. Between the 65% and 67% identity we observed different behaviors suggesting
30 that other factors influence the cross-hybridization (e.g. the size of the identical regions,
31 the percentage of cytosine or guanine nucleotides, etc.).

1 Next, we evaluated the cross-reactivity of the three polyprobes (GP3, GFP5 and
2 GP7) (Figure 4). For this purpose, we used replicas of the same membrane described in
3 Figure 3 that were hybridized at 50°C, since no cross-hybridization was observed with
4 the negative controls at this temperature. First, we observed that the shorter polyprobe
5 carrying only three sequences (GP3: PPV, SPFMV, TEV) was able to detect the 7
6 potyvirus sequences in which the lower detection limit corresponded to the PVMV
7 transcripts at 8 pg/μl (Figure 4). The inclusion of another two viral sequences in the
8 GP5 polyprobe (PVY, PVMV, PPV, SPFMV, TEV) allowed the detection of all 7 viral
9 sequences with lower detection limits, at least for the sequences present in the
10 polyprobe. Thus, we observed positive hybridization signal for all sequences included in
11 the GP5 until a concentration of 0.06 pg/μl, meanwhile the other two sequences not
12 present in the polyprobe were detected until a concentration 1.6 pg/μl, five times less
13 sensitive than the GP3. Apparently, the enlargement of the polyprobe has a slight
14 negative effect in the detection limit of the heterologous sequences. Finally, we
15 hybridized the membrane with the GP7 polyprobe and we obtained the same detection
16 limit for all analyzed viruses, corresponding to 0.32 pg/μl. As observed for the GP5, the
17 increment of the size of the polyprobe affected the detection limit, reducing five times
18 the best signals obtained with GP3 or GP5.

19

20 ***In silico* analysis of the capacity of GP to detect all potyviruses available at the** 21 **database and other members of the family *Potyviridae***

22 According to the results obtained, sequences showing an identity percentage of
23 68% or higher with the selected probes, could be detected by cross-hybridization. To
24 identify how many potyvirus sequences could be potentially hybridized with the cloned
25 potyvirus sequences, we performed a MatGAT analysis (Campanella et al., 2003) using
26 the equivalent region of the 94 potyvirus species used for the phylogenetic analysis
27 (Supplementary figure 2). The results showed that all potyvirus species presented an
28 identity percentage of 68% or higher with two or more of the 7 selected sequences. Only
29 *Onion yellow dwarf virus*, with a percentage of 69.3%, showed such identity with only
30 PPV. In addition, we observed that the majority of the sequences presented identity
31 percentages of 70% or higher with any of the selected sequences. Only *Daphne mosaic*
32 *virus*, *Habenaria mosaic virus*, *Hordeum mosaic virus*, *Onion yellow dwarf virus*,

1 *Ornithogalum mosaic virus* and *Vallota speciosa virus* presented identity percentages
2 below 70%. Also, we observed that the 94 potyvirus species could be theoretically
3 detected with the smaller genus-probe of GP3, since all sequences presented an identity
4 percentage higher than 68% with any of the PPV, TEV and SPFMV cloned fragments.

5 To identify if other members of the family *Potyviridae* could be potentially
6 detected with the seven selected potyvirus sequences, the equivalent regions from 27
7 species representatives of the seven genera of the family *Potyviridae*, except Potyvirus
8 (Supplementary Figure 1), were subjected to a MatGAT analysis (Campanella et al.,
9 2003) to determine the identity percentage (Supplementary Figure 2). The results
10 obtained revealed that all viruses, except Rymovirus, presented an identity percentage
11 below 65%, suggesting that they should be not detected with any of the different probes
12 assayed herein. In the case of rymovirus, the three species analyzed showed identity
13 percentage of 68% or higher with one or more of the 7 selected sequences and, thus,
14 susceptible of being detected by the GPs.

15

16 **Analysis of field samples by non-radioactive nucleic acids spot hybridization** 17 **(NASH)**

18 Finally, we evaluated the capacity of the three GP to detect heterologous
19 potyviruses or other viral species of the family *Potyviridae* by analyzing 49 different
20 field samples, including different hosts, for the presence of 32 potyvirus species or 7
21 viral species assigned to the genus *Bymovirus*, *Ipomovirus*, *Rymovirus* and *Tritimovirus*
22 (Table 2). First, it should be mentioned that the infected starting material was
23 lyophilized or fresh tissue proportionated by the German Collection of Microorganisms
24 and Cell Cultures (DSMZ) or the Mediterranean Agroforestral Institute of the
25 Polytechnic University of Valencia. The samples were extracted using the Silica
26 protocol (MacKenzie et al., 1997) but also with a fast protocol in which the tissue is
27 homogenized with citrate buffer and directly applied onto the membrane (Sanchez-
28 Navarro et al., 1998, Sanchez-Navarro et al., 1999). First, we observed no hybridization
29 signal with any of the 8 healthy hosts analyzed, in spite of that the hybridization was
30 performed at 50°C. The 32 potyviruses and the 2 rymoviruses were detected by NASH

1 with the three GPs except for PVMV that rendered negative signal with GP3.
2 Interestingly, GP3 presents the most unfavorable situation for PVMV detection since its
3 identity percentage with the three cloned sequences present in GP3 is around 66-68%
4 (Figure 3 and 5). On the other hand, we observed that *Onion yellow dwarf virus*, which
5 theoretically could be detected only with the PPV clone (identity percentage of 69.3%),
6 was detected with all three GPs. In addition, *Ryegrass mosaic virus* was clearly detected
7 with GP3 and GP5, in spite that the higher identity percentage in both probes
8 corresponded to the PPV cloned sequence with a 67.2%. When the plants were analyzed
9 using the fast citrate buffer protocol, we observed that the 90.2% of Silica positives (83
10 out 92) were correctly detected. We also observed two samples that were positive by
11 citrate buffer extraction and negative by Silica extraction, using the GP3 and GP5
12 probes (samples 30 and 34, Table 2). These results could indicate differences in the viral
13 titer associated to the extraction protocol used but we cannot discard other effects
14 derived of the starting material (lyophilized) or the host (*Nicotiana benthamiana* or
15 *Cucurbita pepo*) that could interfere with the detection procedure.

16

17 DISCUSSION

18 In the last few years, a significant effort has been made to develop strategies for
19 simultaneous detection of multiple plant viruses. Although PCR-based approaches have
20 received special attention, molecular hybridization assay represents an attractive
21 methodology for the detection of plant viruses since it is accurate, sensitive (pg/ μ l of
22 viral RNA), robust and very powerful for large screening with a reduced cost (see James
23 et al., 2006; Pallás et al., 2011; 2017 for review). Previous works showed that this
24 technology allows the multiple detection of plant viruses by a cocktail of specific probes
25 or by a unique probe carrying the different viral fragments fused in tandem called a
26 polyprobe. The polyprobes permit the detection of several pathogens with comparable
27 detection limit to the individual probes (e.g. Herranz et al., 2005) although with long
28 polyprobes (e.g. 10 probes in tandem or more) a reduction of the hybridization
29 temperature is required (e.g. Peiró et al., 2012). A challenge of this strategy has been its
30 adaptation by generating a polyvalent polyprobe able to detect all the species within a
31 genus, named genus-probe. To answer this question we have selected the genus
32 *Potyvirus* for three reasons: i) it is one of the largest groups of plant viruses including

1 146 species (Revers and Garcia, 2015), ii) potyviruses affect many species which are
2 economically important (Scholthof et al., 2011) and iii) some other diagnosis assays
3 have been adjusted for the detection of a broad spectrum of potyviruses with varied
4 success (Liu et al., 2015, Wei et al., 2009, Hsu et al., 2005, Chen et al., 2001; Jordan
5 and Hammond, 1991). To obtain the genus-probe we have used the versatility of the
6 molecular hybridization that permits the detection of closely related sequences by
7 reducing the hybridization temperature. Thus, we selected the more conserved region,
8 representing 500 nt of the N1b gene, of seven potyviruses distributed along the genus
9 phylogenetic tree and we observed that cross-hybridization occurs at 55°C (single
10 probes) or 50°C (polyprobes) when the target sequences share a 68% or higher identity
11 percentage with the probe, meanwhile negative hybridization signal was obtained when
12 the percentage was below 65%. We observed some exceptions to these rules (e.g. LMV
13 is not detected by PPV probe sharing 69%) suggesting that factors other than the
14 identity percentage are important in the cross-hybridization. In this sense, the presence
15 of large identical regions, the percentage of cytosine and guanines or the probe
16 mismatches, among others, are critical factors to take into account (Kessler, 2000). In
17 these hybridization conditions, there were no positive signals in the different healthy
18 controls used, opening the applicability of this assay for routine diagnosis. According
19 with the identity percentage required for cross-hybridization, the three GP described
20 herein have the capacity to detect the 94 potyvirus species analyzed. However, we
21 observed some negative results (samples 29, 30, or 33) using the GP3 that were positive
22 by GP5 or GP7. Apparently, the presence of more conserved fragments with the capacity
23 to hybridize with a target sequence increments the detection limit of the genus-probe. In
24 our hands, we were able to detect 100% of potyviruses analyzed (32 species in 34
25 samples), representing a comparable (33 species in 55 samples; Jordan and Hammond,
26 1991) or a significant increment from previous approaches using broad-spectrum
27 monoclonal antibody (14 species; Liu et al., 2015) or polyvalent primer pairs that
28 ranged between the 100% (23 species in 40 samples; Zheng et al., 2010), 80% (32 out
29 40 samples corresponding to 21 species out 23; Pappu et al., 1993) or 50% (20 out 40
30 samples corresponding to 15 species out 23; Gibbs and Mackenzie, 1997). We also
31 observed a positive hybridization signal with the two rymovirus analyzed, meanwhile
32 no hybridization was observed with the rest of viral species assigned to the genus
33 bymovirus, ipomovirus or tritimovirus. Interesting, the two rymovirus AgMV and
34 RGMV (samples 39, 40) plus BrSMV (tritimovirus, sample 41) and BaYMV

1 (bymovirus, sample 25) were also detected by RT-PCR using the universal potyviruses
2 NIb primers (Zhen et al., 2010) (data not shown), indicating high conserved sequences
3 in this region in the family *Potyviridae*. Positive hybridization signal was also observed
4 in some species that share only an identity percentage close to the estimated limit of
5 68% for cross-hybridization, with one of the conserved fragments of the GP (e.g. *Onion*
6 *yellow dwarf virus* or *Ryegrass mosaic virus*). In addition, the possibility to use a fast
7 protocol, which detected the 90% of positive samples analyzed by NASH, together with
8 the robustness of the methodology that permits the detection of species with significant
9 nucleotide sequences variability or even uncharacterized potyviruses and the reduced
10 time and cost make the non-radioactive hybridization using a GP a very suitable
11 approach for the routine diagnosis of potyvirus in large surveys. Currently, the use of a
12 broad-spectrum MAb PTY1 (Jordan and Hammond, 1991), combined with a direct
13 tissue extract procedure, represents a valuable tool for diagnostic and screening
14 applications for the detection of aphid-transmissible potyviruses, although it is not
15 known how large is the spectrum of viruses that could be detected by the MAb PTY1.
16 The genus-probed developed in this work anticipates that all potyviruses can be broadly
17 detected (identity percentage of 68/69% or higher). Furthermore, this technology
18 presents the advantage to be easily updated by introducing new sequences of interest
19 (e.g. new potyviruses, etc.). In addition, the use of this new GP, in conjunction to other
20 molecular techniques that permit the identification of the corresponding potyvirus
21 species (e.g. degenerate primer-based RT-PCR or HTS methods, etc.), could be a very
22 good approach to detect any potyvirus isolate, previously described or uncharacterized.
23 Finally, the observation that the GP could detect also members of the genus *Rymovirus*,
24 opens the possibility to design GP against the family *Potyviridae*. An open question
25 related to the GP technology is the size of the conserved regions to be introduced in the
26 polyprobe. In the present work we have selected fragments of around 500 nt but further
27 analysis will be addressed to delimitate the minimal size of the conserved regions that
28 still are able to cross-hybridize.

29

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 3 Polytechnic University of Valencia, for provide part of the potyvirus infected field
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6
 7
 8
 9
 10 **Figure 1.** Phylogenetic trees of the 94 potyviruses for which the complete sequence is
 11 known. Trees were developed using nucleotide sequences of the complete genome and
 12 inferred by the minimum-evolution method. Values at the nodes are bootstrap values
 13 based on 10,000 pseudoreplicates. Nodes with bootstrap support <50% are not
 14 indicated. Sequences in gray boxes were used for the synthesis of the genus-probes.
 15 Sequences underlined correspond to the potyvirus used for the hybridization detection
 16 using the genus-probes.

17
 18 **Figure 2.** Schematic representation of the different genus-probes (GP) 3, 5 and 7 clones
 19 introduced in the pSK+ plasmid. A) The corresponding virus fragments are indicated by
 20 a box in which the numbers represent the corresponding nucleotide of the data base
 21 sequences: X97704 (*Lettuce mosaic virus*, LMV), EU660589 (*Watermelon mosaic*
 22 *virus*, WMV), AJ890346 (*Potato virus Y*, PVY), DQ645484 (*Pepper veinal mottle*
 23 *virus*, PVMV), KU508427 (*Plum pox virus*, PPV), KU511268 (*Sweet potato feathery*
 24 *mottle virus*, SPFMV) and DQ986288 (*Tobacco etch virus*, TEV). The *Xho*I restriction
 25 site used either to synthesize the riboprobe with the T7 RNA polymerase or to insert
 26 additional cDNA probes is indicated. B) Schematic representation of the identity

1 percentage estimated by MatGAT between the sequences used for the genus-probes
2 LMV, PPV, PVMV, PVY, SPFMV, TEV and WMV.

3

4 **Figure 3.** Evaluation of the cross hybridization between the seven potyvirus sequences
5 indicated in Figure 2. A) Influence of the temperature in the cross hybridization between
6 the PPV probe and the rest of the indicated potyvirus sequences. Known amounts of the
7 positive transcripts corresponding to the sequences indicated in Figure 2, were applied
8 on nylon membranes. For the healthy control 100 ng of total RNA extracted from melon
9 (A), cucumber (B), *N. benthamiana* (C), tomato (D), chrysanthemum (E) and gynura
10 (F), were applied. Replicas of the same membrane were hybridized with the PPV
11 riboprobe, complementary to the sequence indicated in Figure 2, at 68°C, 60°C, 55°C
12 and 50°C, respectively. Numbers on the top of the left panel indicate the picograms of
13 transcripts applied on the membrane meanwhile numbers on the right side indicate the
14 identity percentage estimated by the MatGAT program (Campanella et al., 2003)
15 between the PPV sequence and the rest of the potyvirus sequences indicated in Figure 1.
16 B). Summary of the results obtained in A. Numbers represent the identity percentage
17 between the PPV sequence and the rest of the potyvirus sequences meanwhile the colors
18 indicate the lowest amount for which transcripts showed hybridization with the PPV
19 probe. The colors used are blue (200 pg), dark blue (40 pg), green (8 pg), yellow (1.6
20 pg), orange (0.32 pg) and red (0.06 pg). White color indicates no hybridization signal.
21 C) Schematic representation of the hybridization observed with the seven individual
22 potyvirus probes. Replicas of the membrane described in A were hybridized at 55 °C
23 with the indicated probes. Numbers represent the corresponding identity percentage
24 estimated by MatGAT program meanwhile the colors represent the lowest amount of
25 transcripts showing hybridization with the corresponding probe, as indicated in B. Films
26 were exposed for 15 min.

27 **Figure 4.** Evaluation of the cross hybridization between the seven potyvirus sequences
28 and the genus-probes (GP) 3, 5 and 7. A) Replicas of the membrane described in Figure
29 3A were hybridized with the indicated genus-probe at 50°C. For the healthy control 100
30 ng of total RNA extracted from melon (A), cucumber (B), *N. benthamiana* (C), tomato
31 (D), chrysanthemum (E) and gynura (F) were applied. Numbers on the top of the left
32 panel indicate the picograms of transcripts applied on the membrane. Films were

1 exposed for 15 min. B) Summary of the results obtained in A. The colors indicate the
2 lowest amount of transcripts showing hybridization with the corresponding genus-
3 probe. The colors used are blue (200 pg), dark blue (40 pg), green (8 pg), yellow (1.6
4 pg), orange (0.32 pg) and red (0.06 pg). White color indicates no hybridization signal.

5 **Supplementary Figure 2.** Schematic representation of the identity percentage
6 estimated by MatGAT between the sequences used for the genus-probes of LMV, PPV,
7 PVMV, PVY, SPFMV, TEV, WMV and the equivalent sequence of the indicated
8 potyvirus, brambyvirus, bymovirus, ipomovirus, macluravirus, poacevirus, rymovirus
9 and tritimovirus. Identity percentage values of 68% and 69% are labelled in green
10 meanwhile values of 70% and higher are marked in orange. Sequences in gray boxes
11 correspond to the virus used for the hybridization detection using the genus-probes.

12

13

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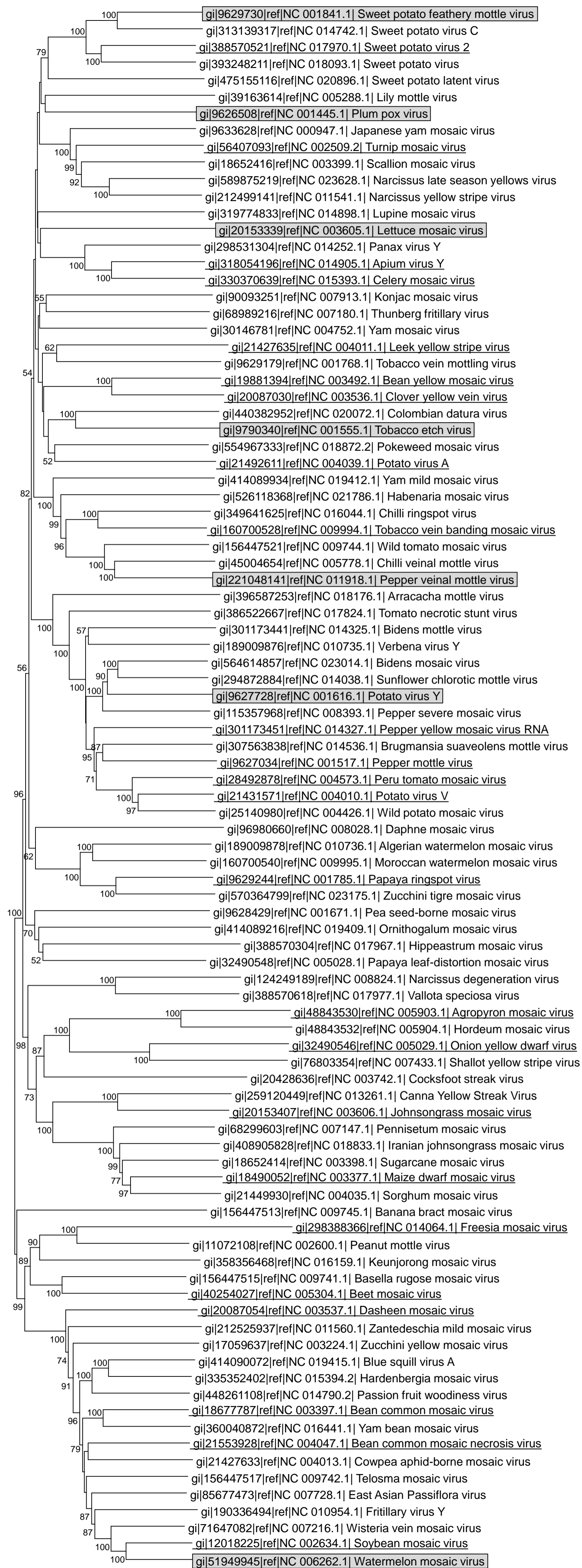
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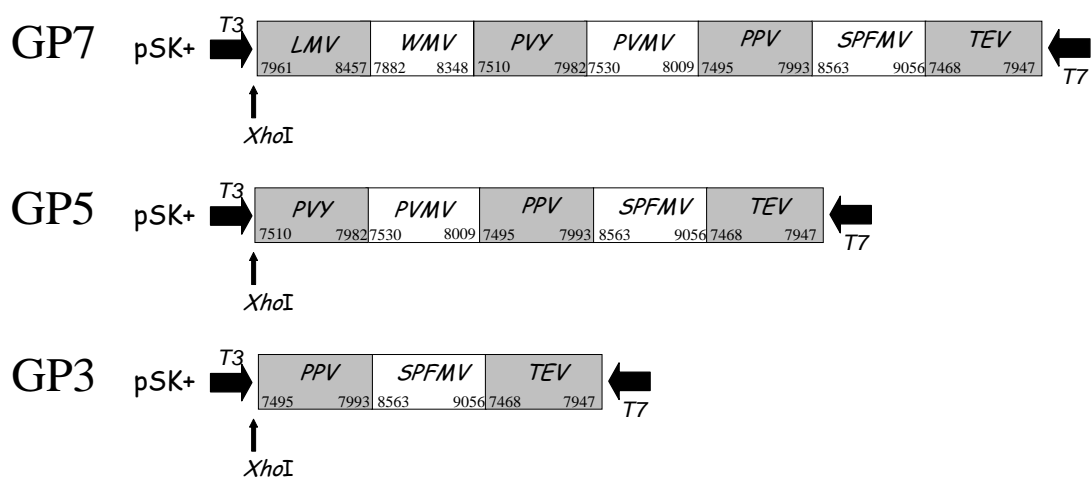
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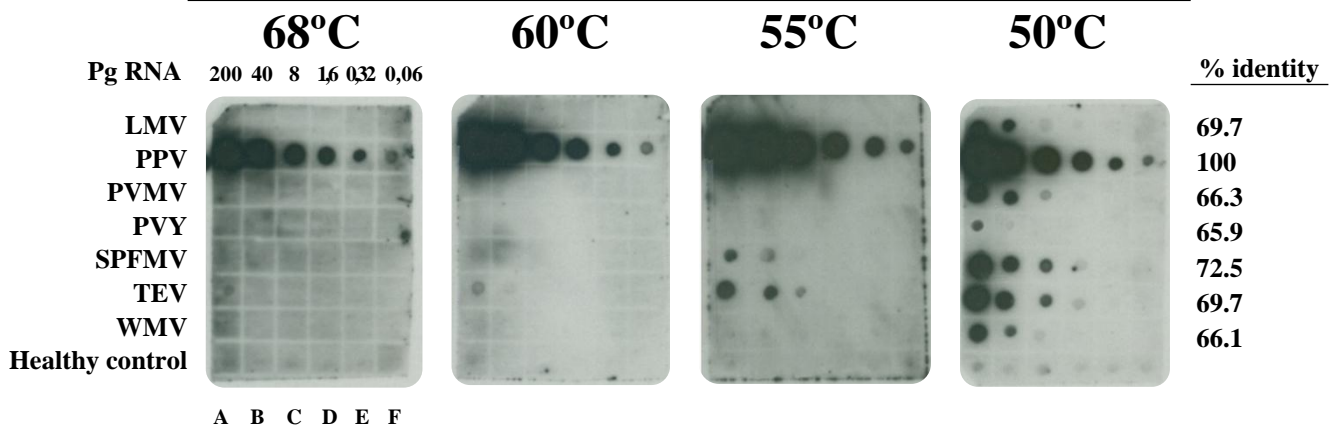
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A**B**

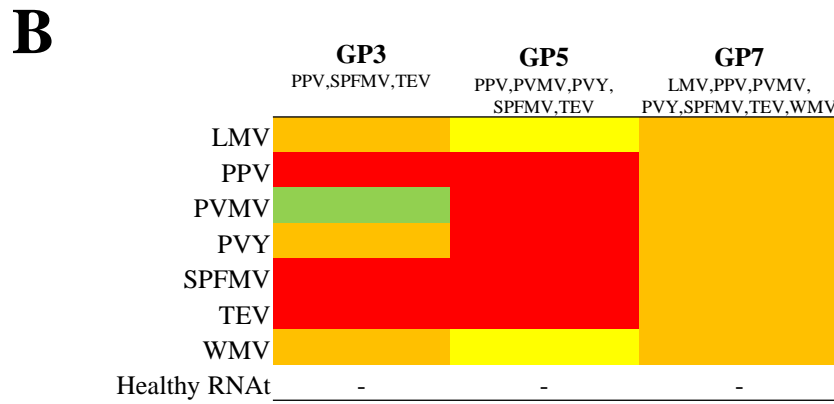
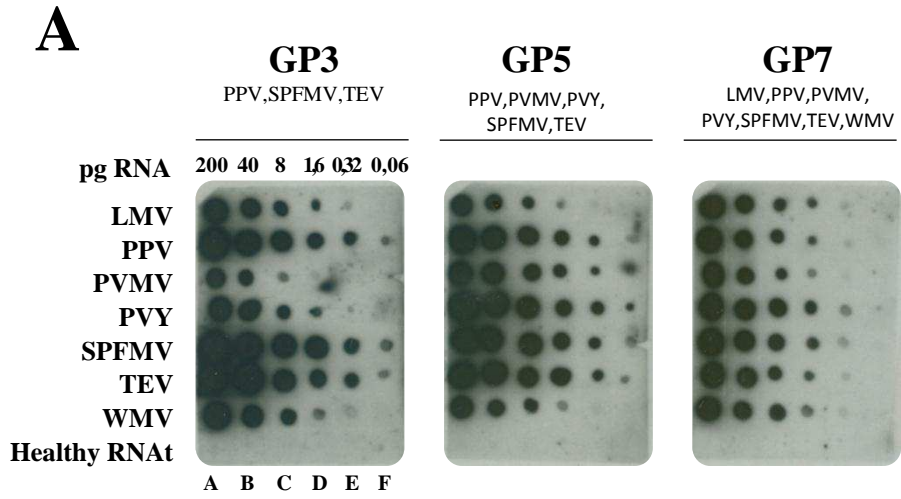
| | LMV | PPV | PVMV | PVY | SPFMV | TEV | WMV |
|-------|------|------|------|------|-------|------|-----|
| LMV | 100 | | | | | | |
| PPV | 69.7 | 100 | | | | | |
| PVMV | 67.6 | 66.3 | 100 | | | | |
| PVY | 64.8 | 65.9 | 66.5 | 100 | | | |
| SPFMV | 69.6 | 72.5 | 66.0 | 67.0 | 100 | | |
| TEV | 67.8 | 69.7 | 66.7 | 69.2 | 67.8 | 100 | |
| WMV | 65.8 | 66.1 | 66.1 | 67.3 | 67.6 | 70.3 | 100 |

A**PPV probe****B****PPV probe**

| | PPV probe | | | |
|-----------------|------------------|--------|--------|--------|
| | (68°C) | (60°C) | (55°C) | (50°C) |
| LMV | 69.7 | 69.7 | 69.7 | 69.7 |
| PPV | 100 | 100 | 100 | 100 |
| PVMV | 66.3 | 66.3 | 66.3 | 66.3 |
| PVY | 65.9 | 65.9 | 65.9 | 65.9 |
| SPFMV | 72.5 | 72.5 | 72.5 | 72.5 |
| TEV | 69.7 | 69.7 | 69.7 | 69.7 |
| WMV | 66.1 | 66.1 | 66.1 | 66.1 |
| Healthy control | - | - | - | + |

C**Probe (55°C)**

| | LMV | PPV | PVMV | PVY | SPFMV | TEV | WMV |
|-----------------|------|------|------|------|-------|------|------|
| LMV | 100 | 69.7 | 67.6 | 64.8 | 69.6 | 67.8 | 65.8 |
| PPV | 69.7 | 100 | 66.3 | 65.9 | 72.5 | 69.7 | 66.1 |
| PVMV | 67.6 | 66.3 | 100 | 66.5 | 66.0 | 66.7 | 66.1 |
| PVY | 64.8 | 65.9 | 66.5 | 100 | 67.0 | 69.2 | 67.3 |
| SPFMV | 69.6 | 72.5 | 66.0 | 67.0 | 100 | 67.8 | 67.6 |
| TEV | 67.8 | 69.7 | 66.7 | 69.2 | 67.8 | 100 | 70.3 |
| WMV | 65.8 | 66.1 | 66.1 | 67.3 | 67.6 | 70.3 | 100 |
| Healthy control | - | - | - | - | +/- | - | - |



| Primer name | Nucleotide sequence (5'-3') ^a | Target viral species | Expected Fragment (bp) | Acc. No. and location in the genome ^b |
|-------------|--|---|------------------------|--|
| 2847-s | CACACTCGCGTAAGTTTGGAGTGTGGAA | <i>Lettuce mosaic virus</i> , LMV | 496 | X97704 |
| 2848-As | CACAGTCGACGTCATAGCTAGCACCAACCAT | | | 7961-8457 |
| 2869-s | CACACTCGAGAAGGGAATTTGGAAYGGTTC | <i>Watermelon mosaic virus</i> , WMV | 466 | EU660589 |
| 2870-As | CACAGTCGACTTGTCACGACTGTAGATGG | | | 7882-8348 |
| 2845-s | CACACTCGAGATTTGGAACGGATCATTGAA | <i>Potato virus Y</i> , PVY | 472 | AJ890346 |
| 2846-As | CACAGTCGACACCATGAGAGAATTATCCAC | | | 7510-7982 |
| 2865-s | CACACTCGAGGTAACCTTGGGATTTGG | <i>Pepper veinal mottle virus</i> , PVMV | 479 | DQ645484 |
| 2866-As | CACAGTCGACCATGAGAGTGTTATC | | | 7530-8009 |
| 2871-s | CACACTCGAGGAAAGAAAGGAGTGTGGAATGG | <i>Plum pox virus</i> , PPV | 498 | KU508427 |
| 2872-As | CACAGTCGCGTCATTGCCAAAATAACCAT | | | 7495-7993 |
| 2873-s | CACACTCGAGATGGGATACAAAAGTCTYTGGAA | <i>Sweet potato feathery mottle virus</i> , SPFMV | 493 | KU511268 |
| 2874-As | CACAGTCGACGCTAACACAACCATAAGTGT | | | 8563-9056 |
| 2861-s | CACACTCGAGGAAAGCTGGGAATTTGG | <i>Tobacco etch virus</i> , TEV | 479 | DQ986288 |
| 2862-As | CACAGTCGACCATGAGTGTGTTGTC | | | 7468-7947 |

Table 1. Primer pairs used in the amplification of the genus-probes.

^a Restriction sites of the *XhoI* and *SaI* are underlined.^b Numbers are referred to the corresponding nucleotide of the sequence available in the indicated accession number of the GenBank database.

Table 2. Analysis of the capacity of the genus-probes (GP) 3, 5 and 7 to detect different potyvirus, bymovirus, ipomovirus, rymovirus and tritimovirus in field samples by nucleic acids spot hybridization (NASH). All samples were extracted with the Silica and the citrate buffer protocols. The hybridization was performed at 50°C.

| | Virus | Acronym | Genus | Source ^a | Host | NASH (Silica/Citrate buffer) | | |
|----|-----------------------------------|---------|------------------|---------------------|-------------------------------|---------------------------------|------|------|
| | | | | | | GP3 | GP5 | GP7 |
| 1 | Carrot virus Y | CarVY | <i>Potyvirus</i> | UPV | <i>Daucus carota</i> | +/+ | +/+ | +/+ |
| 2 | Onion yellow dwarf virus | OYDV | <i>Potyvirus</i> | UPV | <i>Allium cepa</i> | +/- | +/- | +/+ |
| 3 | Turnip mosaic virus | TuMV | <i>Potyvirus</i> | UPV | <i>Lactuca sativa</i> | +/+ | +/+ | +/- |
| 4 | Bean common mosaic necrosis virus | BCMNV | <i>Potyvirus</i> | DSMZ | <i>Phaseolus vulgaris</i> | +/+ | +/+ | +/+ |
| 5 | Bean common mosaic virus | BCMV | <i>Potyvirus</i> | DSMZ | <i>Phaseolus vulgaris</i> | +/+ | +/+ | +/+ |
| 6 | Beet mosaic virus | BtMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 7 | Johnsongrass mosaic virus | JGMV | <i>Potyvirus</i> | DSMZ | <i>Zea mays</i> | +/+ | +/+ | +/+ |
| 8 | Leek yellow stripe virus | LYSV | <i>Potyvirus</i> | DSMZ | <i>Chenopodium quinoa</i> | +/+ | +/+ | +/+ |
| 9 | Pepper mottle virus | PepMoV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana tabacum</i> | +/* | +/+ | +/+ |
| 10 | Peru tomato mosaic virus | PTV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana tabacum</i> | +/* | +/+ | +/+ |
| 11 | Potato virus A | PVA | <i>Potyvirus</i> | DSMZ | <i>Nicotiana occidentalis</i> | +/+ | +/+ | +/+ |
| 12 | Potato virus V | PVV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 13 | Potato virus Y | PVY | <i>Potyvirus</i> | DSMZ | <i>Nicotiana tabacum</i> | +/* | +/+ | +/- |
| 14 | Soybean mosaic virus | SMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/* |
| 15 | Tobacco vein banding mosaic virus | TVBMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 16 | Maize dwarf mosaic virus | MDMV | <i>Potyvirus</i> | DSMZ | <i>Zea mays</i> | +/+ | +/+ | +/+ |
| 17 | Dasheen mosaic virus | DsMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 18 | Apium virus Y | ApVY | <i>Potyvirus</i> | DSMZ | <i>Ami majus</i> | +/+ | +/+ | +/+ |
| 19 | Pepper yellow mosaic virus | PepYMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana tabacum</i> | +/* | +/+ | +/+ |
| 20 | Sweet potato virus 2 | SPV-2 | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 21 | Bean yellow mosaic virus | BYMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 22 | Carrot thin leaf virus | CTLV | <i>Potyvirus</i> | UPV | <i>Daucus carota</i> | +/+ | +/+ | +/+ |
| 23 | Celery mosaic virus | CeMV | <i>Potyvirus</i> | UPV | <i>Apium graveolens</i> | +/nt | +/nt | +/nt |
| 24 | Clover yellow vein virus | CIYVV | <i>Potyvirus</i> | UPV | <i>Phaseolus vulgaris</i> | +/nt | +/nt | +/nt |
| 25 | Freesia mosaic virus | FreMV | <i>Potyvirus</i> | UPV | <i>Freesia</i> | +/+ | +/+ | +/- |
| 26 | Papaya ringspot virus | PRSV | <i>Potyvirus</i> | UPV | <i>Citrullus lanatus</i> | +/nt | +/nt | +/nt |

| | | | | | | | | |
|----|------------------------------------|-------|---------------------|------|------------------------------|-------|-------|-------|
| 27 | Tobacco etch virus | TEV | <i>Potyvirus</i> | UPV | <i>Nicotiana tabacum</i> | +/+ | +/+ | +/+ |
| 28 | Tobacco etch virus | TEV | <i>Potyvirus</i> | UPV | <i>Physalis floridana</i> | +/+ | +/+ | +/+ |
| 29 | Lettuce mosaic virus | LMV | <i>Potyvirus</i> | DSMZ | <i>Chenopodium quinoa</i> | -/+ | -/+ | +/+ |
| 30 | Pepper veinal mottle virus | PVMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | -/- | +/+ | +/+ |
| 31 | Sweet potato feathery mottle virus | SPFMV | <i>Potyvirus</i> | DSMZ | <i>Nicotiana benthamiana</i> | +/+ | +/+ | +/+ |
| 32 | Tobacco etch virus | TEV | <i>Potyvirus</i> | UPV | <i>Nicotiana tabacum</i> | +/+ | +/+ | +/+ |
| 33 | Watermelon mosaic virus | WMV | <i>Potyvirus</i> | DSMZ | <i>Cucurbita pepo</i> | -/+ | -/+ | +/+ |
| 34 | Plum pox virus | PPV | <i>Potyvirus</i> | Lab | <i>Prunus domestica</i> | + /nt | + /nt | + /nt |
| 35 | Barley yellow mosaic virus | BaYMV | <i>Bymovirus</i> | DSMZ | <i>Hordeum vulgare</i> | -/- | -/- | -/- |
| 36 | Ugandan cassava brown streak virus | UCBSV | <i>Ipomovirus</i> | DSMZ | <i>Manihot esculenta</i> | -/- | -/- | -/- |
| 37 | Cucumber vein yellowing virus | CVYV | <i>Ipomovirus</i> | UPV | <i>Cucumis sativus</i> | -/- | -/- | -/- |
| 38 | Sweet potato mild mottle virus | SPMMV | <i>Ipomovirus</i> | DSMZ | <i>Nicotiana tabacum</i> | -/- | -/- | -/- |
| 39 | Agropyron mosaic virus | AgMV | <i>Rymovirus</i> | DSMZ | <i>Triticum aestivum</i> | +/+ | +/+ | +/+ |
| 40 | Ryegrass mosaic virus | RGMV | <i>Rymovirus</i> | DSMZ | <i>Lolium multiflorum</i> | +/+ | +/+ | +/+ |
| 41 | Brome streak mosaic virus | BrSMV | <i>Tritimovirus</i> | DSMZ | <i>Hordeum vulgare</i> | * /- | * /- | * /- |
| 42 | Healthy control | | | Lab | <i>Cucumis melo</i> | - /nt | - /nt | - /nt |
| 43 | Healthy control | | | Lab | <i>Cucumis sativus</i> | - /nt | - /nt | - /nt |
| 44 | Healthy control | | | Lab | <i>Nicotiana benthamiana</i> | - /- | - /- | - /- |
| 45 | Healthy control | | | Lab | <i>Solanum lycopersicum</i> | - /nt | - /nt | - /nt |
| 46 | Healthy control | | | Lab | <i>Chrysanthemum</i> | - /nt | - /nt | - /nt |
| 47 | Healthy control | | | Lab | <i>Gynura aurantiaca</i> | - /nt | - /nt | - /nt |
| 48 | Healthy control | | | Lab | <i>Nicotiana tabacum</i> | - /- | - /- | - /- |
| 49 | Healthy control | | | UPV | <i>Arabidopsis thaliana</i> | - /- | - /- | - /- |

+, - and * correspond to positive, negative and uncertain results, respectively.

nt, no tissue available.

^a, samples obtained from the Isabel Font's group at the Universidad Politécnica de Valencia (UPV), the German Collection of Microorganisms and Cell Cultures (DSMZ) or our laboratory (Lab).

Conserved regions between viral species belonging to the family *Potyviridae*

>gi|48843530|ref|NC_005903.1|_Agropyron_mosaic_virus_complete_genome

AAAGTTTGGTGTATGGAACGGATCGTTGAAAGCTGAGCTTAGACCAAATGCAAAGGTTGAAGCGAACA
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GCTCATTAACTCTTATCTCATAAACGCCATTCTCAATGTTGATTGCAGTTCATGCAGGAATGGAACAT
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AGTCATTAAGAAGTTCAGAGGGAACAATAGCGGACAGCCATCAACAGTCGTTGACAATACATTAATGG
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>gi|189009878|ref|NC_010736.1|_Algerian_watermelon_mosaic_virus_complete_genome

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TGGGATGAGTTTTGCGAAAGTTCCAGATAATTGGGTTTACTGTGATGCTGATGGGTCTCAGTTTGT
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CAATCGTCAAGAAATTCAAAGGCAATAACAGTGGTCAACCGTCAACAGTTGTAGATAATACGCTCATGG
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>gi|318054196|ref|NC_014905.1|_Apium_virus_Y_complete_genome

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>gi|396587253|ref|NC_018176.1|_Arracacha_mottle_virus_complete_genome

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ACAGTGCTAAAGAAATTTAGAGGGAACAATAGTGGACAGCCCTCAACGGTTGTTGATAATTCTTTGATG
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>gi|156447513|ref|NC_009745.1|_Banana_bract_mosaic_virus_complete_genome

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>gi|156447515|ref|NC_009741.1|_Basella_rugose_mosaic_virus_complete_genome

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>gi|21553928|ref|NC_004047.1|_Bean_common_mosaic_necrosis_virus_complete_genome

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>gi|18677787|ref|NC_003397.1|_Bean_common_mosaic_virus_complete_genome

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>gi|19881394|ref|NC_003492.1|_Bean_yellow_mosaic_virus_complete_genome

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 GCTCACTCACTCCATACCTTTTGAATGCTGCTTGTGATGCGTTGAGACTTATGGAAGAATGGGATT
 TGGGTGAACAGATGTTGAAAAATCTTTATACAGAAATTGTGTACACACCCATATTGACACCAGATGGAA

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>gi|40254027|ref|NC_005304.1|_Beet_mosaic_virus_complete_genome

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>gi|564614857|ref|NC_023014.1|_Bidens_mosaic_virus_isolate_SP01_complete_genome

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>gi|301173441|ref|NC_014325.1|_Bidens_mottle_virus_complete_genome

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>gi|414090072|ref|NC_019415.1|_Blue_squill_virus_A_complete_genome

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>gi|307563838|ref|NC_014536.1|_Brugmansia_suaveolens_mottle_virus_complete_genome

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>gi|259120449|ref|NC_013261.1|_Canna_Yellow_Streak_Virus_complete_genome

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>gi|330370639|ref|NC_015393.1|_Celery_mosaic_virus_complete_genome

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 GGTGAGAAGATGCTATCAAATTTGTACACAGAAATAGTCTACACACCCATTCTCACGCCAGATGGAACG
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>gi|349641625|ref|NC_016044.1|_Chilli_ringspot_virus_complete_genome

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>gi|45004654|ref|NC_005778.1|_Chilli_veinal_mottle_virus_complete_genome

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>gi|20087030|ref|NC_003536.1|_Clover_yellow_vein_virus_complete_genome

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>gi|20428636|ref|NC_003742.1|_Cocksfoot_streak_virus_complete_genome

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>gi|440382952|ref|NC_020072.1|_Colombian_datura_virus_complete_genome

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>gi|21427633|ref|NC_004013.1|_Cowpea_aphid-borne_mosaic_virus_complete_genome

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>gi|96980660|ref|NC_008028.1|_Daphne_mosaic_virus_complete_genome

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>gi|20087054|ref|NC_003537.1|_Dasheen_mosaic_virus_complete_genome

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>gi|85677473|ref|NC_007728.1|_East_Asian_Passiflora_virus_complete_genome

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>gi|526118368|ref|NC_021786.1|_Habenaria_mosaic_virus_genomic_RNA_complete_genom
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>gi|335352402|ref|NC_015394.2|_Hardenbergia_mosaic_virus_complete_genome

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>NC_024471.1_Yellow_oat-grass_mosaic_virus_isolate_YOgMV-Sb_complete_genome

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>NC_022745.1_Tall_oatgrass_mosaic_virus_isolate_Benesov_complete_genome

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>NC_001886.1_Wheat_streak_mosaic_virus_complete_genome

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ATGTGTTAATCATAGCAATGGA

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This paper has been peer reviewed and accepted for publication but has not yet been copyedited or proofread. The final published version may differ.

| | LMV | PPV | PVMV | PVY | SPFMV | TEV | WMV | Genus |
|---|------|------|------|------|-------|------|------|--------------|
| gi 189009878 ref NC 010736.1 Algerian watermelon mosaic virus | 68.5 | 70.5 | 65.3 | 67.3 | 69.1 | 67.5 | 64.7 | Potyvirus |
| gi 318054196 ref NC 014905.1 Apium virus Y | 72.1 | 73.9 | 68.5 | 64.5 | 73.1 | 66.9 | 66.1 | Potyvirus |
| gi 396587253 ref NC 018176.1 Arracacha mottle virus | 67.7 | 70.3 | 67.7 | 68.5 | 69.9 | 67.1 | 63.7 | Potyvirus |
| gi 156447513 ref NC 009745.1 Banana bract mosaic virus | 69.7 | 67.7 | 60.6 | 68.5 | 70.1 | 69.5 | 64.1 | Potyvirus |
| gi 156447515 ref NC 009741.1 Basella rugose mosaic virus | 68.5 | 68.9 | 67.1 | 68.9 | 70.5 | 65.9 | 62.2 | Potyvirus |
| gi 21553928 ref NC 004047.1 Bean common mosaic necrosis virus | 70.1 | 71.7 | 66.3 | 66.7 | 71.7 | 68.1 | 71.7 | Potyvirus |
| gi 18677787 ref NC 003397.1 Bean common mosaic virus | 68.1 | 69.9 | 63.7 | 65.5 | 71.7 | 68.1 | 70.9 | Potyvirus |
| gi 19881394 ref NC 003492.1 Bean yellow mosaic virus | 71.7 | 71.9 | 67.7 | 68.1 | 71.7 | 69.1 | 68.1 | Potyvirus |
| gi 40254027 ref NC 005304.1 Beet mosaic virus | 71.7 | 71.5 | 68.5 | 67.1 | 70.9 | 69.3 | 64.3 | Potyvirus |
| gi 564614857 ref NC 023014.1 Bidens mosaic virus | 70.1 | 71.1 | 67.5 | 74.9 | 69.7 | 67.1 | 65.5 | Potyvirus |
| gi 301173441 ref NC 014325.1 Bidens mottle virus | 69.1 | 69.3 | 65.9 | 71.5 | 70.3 | 69.3 | 64.7 | Potyvirus |
| gi 414090072 ref NC 019415.1 Blue squill virus A | 69.1 | 69.7 | 65.7 | 66.1 | 71.7 | 69.5 | 72.5 | Potyvirus |
| gi 307563838 ref NC 014536.1 Brugmansia suaveolens mottle virus | 70.9 | 69.9 | 66.9 | 71.5 | 70.3 | 67.3 | 65.5 | Potyvirus |
| gi 259120449 ref NC 013261.1 Canna Yellow Streak Virus | 68.5 | 71.9 | 67.3 | 68.1 | 68.9 | 70.1 | 67.1 | Potyvirus |
| gi 330370639 ref NC 015393.1 Celery mosaic virus | 72.5 | 74.1 | 66.7 | 68.1 | 69.3 | 71.1 | 66.7 | Potyvirus |
| gi 349641625 ref NC 016044.1 Chili ringspot virus | 69.1 | 71.3 | 70.1 | 64.9 | 70.7 | 68.7 | 65.1 | Potyvirus |
| gi 45004654 ref NC 005778.1 Chili vein mottle virus | 68.5 | 69.1 | 72.9 | 64.5 | 66.3 | 65.9 | 62.3 | Potyvirus |
| gi 20087030 ref NC 003536.1 Clover yellow vein virus | 72.1 | 73.1 | 66.9 | 67.3 | 70.3 | 71.1 | 67.3 | Potyvirus |
| gi 20428636 ref NC 003742.1 Cocksfoot streak virus | 70.3 | 68.9 | 64.9 | 67.5 | 66.9 | 67.3 | 62.0 | Potyvirus |
| gi 440382952 ref NC 020072.1 Colombian datura virus | 67.5 | 70.3 | 64.1 | 65.5 | 68.1 | 72.3 | 65.5 | Potyvirus |
| gi 21427633 ref NC 004013.1 Cowpea aphid-borne mosaic virus | 69.1 | 72.3 | 65.5 | 65.7 | 69.7 | 69.3 | 71.9 | Potyvirus |
| gi 96980660 ref NC 008028.1 Daphne mosaic virus | 68.1 | 68.5 | 66.5 | 65.3 | 66.3 | 66.1 | 66.1 | Potyvirus |
| gi 20087054 ref NC 003537.1 Dasheen mosaic virus | 68.1 | 70.3 | 63.7 | 65.7 | 69.7 | 66.9 | 70.7 | Potyvirus |
| gi 85677473 ref NC 007728.1 East Asian Passiflora virus | 68.9 | 70.9 | 66.3 | 66.5 | 71.1 | 68.1 | 71.5 | Potyvirus |
| gi 298388366 ref NC 014064.1 Freesia mosaic virus | 48.9 | 50.5 | 52.0 | 47.6 | 49.5 | 50.4 | 48.0 | Potyvirus |
| gi 190336494 ref NC 010954.1 Fritillary virus Y | 68.9 | 72.3 | 66.1 | 66.1 | 71.3 | 67.7 | 73.9 | Potyvirus |
| gi 526118368 ref NC 021786.1 Habeneria mosaic virus | 66.3 | 69.1 | 68.3 | 64.1 | 68.7 | 67.3 | 63.3 | Potyvirus |
| gi 335352402 ref NC 015394.2 Hardenbergia mosaic virus | 69.3 | 68.7 | 63.1 | 64.5 | 71.5 | 68.3 | 70.9 | Potyvirus |
| gi 388570304 ref NC 017967.1 Hippeastrum mosaic virus | 68.7 | 71.3 | 65.1 | 62.4 | 70.7 | 70.3 | 63.5 | Potyvirus |
| gi 408905828 ref NC 018833.1 Iranian johnsongrass mosaic virus | 71.1 | 69.5 | 67.1 | 67.5 | 68.7 | 69.5 | 66.1 | Potyvirus |
| gi 9633628 ref NC 000947.1 Japanese yam mosaic virus | 70.3 | 71.1 | 67.7 | 66.9 | 71.1 | 72.3 | 65.7 | Potyvirus |
| gi 20153407 ref NC 003606.1 Johnsongrass mosaic virus | 71.1 | 70.7 | 67.1 | 67.9 | 69.3 | 70.1 | 66.7 | Potyvirus |
| gi 358356468 ref NC 016159.1 Keunjongrass mosaic virus | 68.7 | 68.9 | 63.5 | 65.1 | 68.7 | 69.5 | 66.9 | Potyvirus |
| gi 90093251 ref NC 007913.1 Konjac mosaic virus | 68.3 | 71.3 | 65.9 | 66.7 | 70.5 | 69.5 | 60.8 | Potyvirus |
| gi 21427635 ref NC 004011.1 Leek yellow stripe virus | 71.1 | 72.7 | 65.3 | 65.9 | 70.9 | 68.7 | 66.7 | Potyvirus |
| gi 20153339 ref NC 003605.1 Lettuce mosaic virus | 94.0 | 70.3 | 66.5 | 65.1 | 69.5 | 68.1 | 65.1 | Potyvirus |
| gi 39163614 ref NC 005288.1 Lily mottle virus | 67.7 | 71.7 | 65.3 | 67.9 | 70.9 | 70.3 | 64.9 | Potyvirus |
| gi 319774833 ref NC 014898.1 Lupine mosaic virus | 66.3 | 70.3 | 65.7 | 63.9 | 68.1 | 66.1 | 64.3 | Potyvirus |
| gi 18490052 ref NC 003377.1 Maize dwarf mosaic virus | 68.9 | 73.1 | 67.3 | 65.5 | 70.9 | 69.9 | 66.5 | Potyvirus |
| gi 160700540 ref NC 009995.1 Moroccan watermelon mosaic virus | 69.9 | 70.9 | 65.1 | 66.7 | 69.9 | 65.7 | 66.1 | Potyvirus |
| gi 124249189 ref NC 008824.1 Narcissus degeneration virus | 67.3 | 69.9 | 64.7 | 64.3 | 70.3 | 68.1 | 65.1 | Potyvirus |
| gi 589875219 ref NC 023628.1 Narcissus late season yellows virus isolate | 72.3 | 73.7 | 66.9 | 67.1 | 69.5 | 69.9 | 65.5 | Potyvirus |
| gi 212499141 ref NC 011541.1 Narcissus yellow stripe virus | 71.7 | 74.1 | 67.9 | 65.7 | 72.5 | 69.5 | 66.5 | Potyvirus |
| gi 32490546 ref NC 005029.1 Onion yellow dwarf virus | 64.3 | 69.3 | 65.1 | 65.5 | 67.7 | 67.3 | 63.3 | Potyvirus |
| gi 414089216 ref NC 019409.1 Ornithogalum mosaic virus | 68.9 | 69.3 | 67.3 | 63.1 | 67.7 | 69.3 | 62.0 | Potyvirus |
| gi 298531304 ref NC 014252.1 Panax virus Y | 69.3 | 71.1 | 68.7 | 65.5 | 69.9 | 69.9 | 65.1 | Potyvirus |
| gi 32490548 ref NC 005028.1 Papaya leaf-distortion mosaic virus | 67.5 | 67.5 | 65.7 | 66.9 | 69.3 | 70.3 | 68.5 | Potyvirus |
| gi 9629244 ref NC 001785.1 Papaya ringspot virus | 70.1 | 69.7 | 65.7 | 66.5 | 67.7 | 65.1 | 65.7 | Potyvirus |
| gi 448261108 ref NC 014790.2 Passion fruit woodiness virus | 68.1 | 70.7 | 65.3 | 65.5 | 68.5 | 68.9 | 70.5 | Potyvirus |
| gi 9628429 ref NC 001671.1 Pea seed-borne mosaic virus | 69.7 | 69.3 | 65.5 | 65.7 | 71.3 | 68.9 | 65.1 | Potyvirus |
| gi 11072108 ref NC 002600.1 Peanut mottle virus | 70.1 | 69.3 | 65.7 | 66.3 | 72.1 | 68.3 | 66.5 | Potyvirus |
| gi 68299603 ref NC 007147.1 Pennisetum mosaic virus | 70.7 | 73.5 | 66.3 | 67.3 | 68.7 | 69.7 | 64.5 | Potyvirus |
| gi 9627034 ref NC 001517.1 Pepper mottle virus | 70.5 | 70.3 | 66.5 | 69.9 | 71.1 | 67.9 | 63.9 | Potyvirus |
| gi 115357968 ref NC 008393.1 Pepper severe mosaic virus | 70.5 | 71.7 | 68.3 | 70.1 | 69.5 | 65.3 | 67.7 | Potyvirus |
| gi 221048141 ref NC 011918.1 Pepper vein mottle virus | 69.5 | 68.1 | 96.4 | 65.5 | 66.9 | 64.3 | 63.9 | Potyvirus |
| gi 301173451 ref NC 014327.1 Pepper yellow mosaic virus | 69.9 | 70.9 | 66.1 | 72.5 | 71.7 | 70.7 | 65.3 | Potyvirus |
| gi 28492878 ref NC 004573.1 Peru tomato mosaic virus | 71.1 | 72.9 | 65.1 | 70.9 | 67.1 | 70.9 | 65.9 | Potyvirus |
| gi 9626508 ref NC 001445.1 Plum pox virus | 70.3 | 98.4 | 65.9 | 66.3 | 72.1 | 69.7 | 66.3 | Potyvirus |
| gi 554967333 ref NC 018872.2 Pokeweed mosaic virus | 73.9 | 73.5 | 67.1 | 67.1 | 70.3 | 67.5 | 66.5 | Potyvirus |
| gi 21492611 ref NC 004039.1 Potato virus A | 70.9 | 70.7 | 67.1 | 65.9 | 71.7 | 67.7 | 65.1 | Potyvirus |
| gi 21431571 ref NC 004010.1 Potato virus V | 71.5 | 74.3 | 67.5 | 69.5 | 69.1 | 69.5 | 66.9 | Potyvirus |
| gi 9627728 ref NC 001616.1 Potato virus Y | 68.1 | 70.5 | 65.5 | 83.1 | 69.5 | 67.7 | 65.9 | Potyvirus |
| gi 18652416 ref NC 003399.1 Scallion mosaic virus | 70.5 | 73.1 | 70.5 | 65.9 | 71.9 | 71.1 | 67.1 | Potyvirus |
| gi 76803354 ref NC 007433.1 Shallot yellow stripe virus | 69.7 | 70.3 | 67.7 | 64.5 | 68.3 | 68.5 | 67.5 | Potyvirus |
| gi 21449930 ref NC 004035.1 Sorghum mosaic virus | 71.5 | 69.7 | 69.1 | 65.5 | 69.1 | 66.5 | 64.9 | Potyvirus |
| gi 12018225 ref NC 002634.1 Soybean mosaic virus | 67.5 | 73.3 | 66.5 | 65.1 | 71.1 | 69.3 | 76.7 | Potyvirus |
| gi 18652414 ref NC 003398.1 Sugarcane mosaic virus | 70.9 | 69.9 | 66.5 | 68.3 | 66.9 | 70.1 | 65.3 | Potyvirus |
| gi 294872884 ref NC 014038.1 Sunflower chlorotic mottle virus | 71.3 | 69.5 | 65.1 | 72.7 | 71.1 | 68.9 | 65.7 | Potyvirus |
| gi 9629730 ref NC 001841.1 Sweet potato feathery mottle virus | 69.1 | 71.9 | 65.7 | 67.9 | 90.0 | 68.3 | 67.1 | Potyvirus |
| gi 475155116 ref NC 020896.1 Sweet potato latent virus | 69.7 | 73.7 | 66.7 | 67.1 | 71.3 | 72.9 | 64.7 | Potyvirus |
| gi 388570521 ref NC 017970.1 Sweet potato virus 2 | 70.3 | 74.9 | 66.1 | 66.5 | 74.3 | 69.9 | 66.1 | Potyvirus |
| gi 313139317 ref NC 014742.1 Sweet potato virus C | 70.9 | 71.9 | 66.3 | 66.9 | 79.3 | 68.9 | 65.7 | Potyvirus |
| gi 393248211 ref NC 018093.1 Sweet potato virus G | 70.9 | 74.1 | 68.9 | 68.3 | 74.5 | 70.9 | 65.3 | Potyvirus |
| gi 156447517 ref NC 009742.1 Telosma mosaic virus | 69.9 | 70.7 | 67.3 | 65.1 | 69.9 | 67.5 | 73.3 | Potyvirus |
| gi 68989216 ref NC 007180.1 Thunberg fritillary virus | 68.9 | 73.7 | 62.4 | 66.7 | 68.5 | 69.1 | 66.1 | Potyvirus |
| gi 9790340 ref NC 001555.1 Tobacco etch virus | 69.7 | 71.9 | 64.3 | 67.1 | 68.9 | 96.0 | 68.3 | Potyvirus |
| gi 160700528 ref NC 009994.1 Tobacco vein banding mosaic virus | 68.9 | 71.3 | 69.1 | 64.5 | 66.1 | 71.9 | 61.8 | Potyvirus |
| gi 9629179 ref NC 001768.1 Tobacco vein mottling virus | 74.3 | 73.3 | 67.1 | 68.7 | 70.1 | 67.9 | 67.3 | Potyvirus |
| gi 386522667 ref NC 017824.1 Tomato necrotic stunt virus | 72.1 | 72.7 | 68.7 | 70.1 | 72.5 | 70.1 | 68.1 | Potyvirus |
| gi 56407093 ref NC 002509.2 Turnip mosaic virus | 70.5 | 73.1 | 67.1 | 69.1 | 69.5 | 72.5 | 67.1 | Potyvirus |
| gi 388570618 ref NC 017977.1 Vallota speciosa virus | 66.5 | 68.3 | 66.7 | 65.1 | 69.5 | 66.9 | 63.3 | Potyvirus |
| gi 189009876 ref NC 010735.1 Verbena virus Y | 69.9 | 71.3 | 69.5 | 70.3 | 69.3 | 68.1 | 62.7 | Potyvirus |
| gi 51949945 ref NC 006262.1 Watermelon mosaic virus | 69.1 | 71.1 | 65.7 | 66.9 | 71.3 | 69.1 | 92.2 | Potyvirus |
| gi 25140980 ref NC 004426.1 Wild potato mosaic virus | 71.3 | 70.9 | 65.5 | 72.1 | 66.3 | 68.9 | 64.5 | Potyvirus |
| gi 156447521 ref NC 009744.1 Wild tomato mosaic virus | 67.9 | 71.7 | 74.5 | 63.9 | 67.3 | 65.7 | 63.5 | Potyvirus |
| gi 71647082 ref NC 007216.1 Wisteria vein mosaic virus | 68.9 | 70.5 | 67.7 | 67.5 | 73.5 | 70.1 | 74.5 | Potyvirus |
| gi 360040872 ref NC 016441.1 Yam bean mosaic virus | 68.9 | 69.9 | 64.9 | 67.3 | 73.3 | 67.1 | 72.5 | Potyvirus |
| gi 414089934 ref NC 019412.1 Yam mild mosaic virus | 67.5 | 68.1 | 69.7 | 66.9 | 70.7 | 68.5 | 65.9 | Potyvirus |
| gi 30146781 ref NC 004752.1 Yam mosaic virus | 70.7 | 73.3 | 67.7 | 68.7 | 72.3 | 70.1 | 68.7 | Potyvirus |
| gi 212525937 ref NC 011560.1 Zantedeschia mild mosaic virus | 70.5 | 70.1 | 65.9 | 66.5 | 69.1 | 67.3 | 69.9 | Potyvirus |
| gi 570364799 ref NC 023175.1 Zucchini tigre mosaic virus | 67.9 | 69.3 | 64.5 | 65.5 | 71.1 | 66.3 | 64.9 | Potyvirus |
| gi 17059637 ref NC 003224.1 Zucchini yellow mosaic virus | 68.7 | 68.9 | 63.7 | 64.1 | 68.9 | 67.7 | 72.3 | Potyvirus |
| NC 008558.1 Blackberry virus Y | 61.2 | 60.2 | 55.3 | 57.1 | 59.6 | 59.8 | 56.1 | Brambyvirus |
| AY994084.1 Blackberry virus Y | 61.2 | 60.2 | 55.3 | 57.1 | 59.6 | 59.8 | 56.1 | Brambyvirus |
| NC 002350.1 Wheat yellow mosaic virus | 56.6 | 53.6 | 58.2 | 56.2 | 58.6 | 56.0 | 54.4 | Bymovirus |
| NC 004016.1 Oat mosaic virus | 56.2 | 60.0 | 56.6 | 57.2 | 58.8 | 58.8 | 55.0 | Bymovirus |
| NC 002990.1 Barley yellow mosaic virus | 57.1 | 57.1 | 56.5 | 54.3 | 56.7 | 59.6 | 56.7 | Bymovirus |
| NC 028144.1 Rice necrosis mosaic virus | 59.6 | 57.4 | 58.8 | 54.4 | 59.6 | 60.0 | 56.8 | Bymovirus |
| NC 003483.1 Barley mild mosaic virus | 57.2 | 55.0 | 58.0 | 55.4 | 57.0 | 58.6 | 55.4 | Bymovirus |
| NC 030840.1 Coccinia mottle virus | 63.1 | 61.1 | 62.9 | 60.5 | 58.9 | 62.5 | 58.1 | Ipomovirus |
| NC 010521.1 Squash vein yellowing virus | 60.7 | 62.9 | 58.5 | 56.3 | 63.3 | 60.7 | 56.3 | Ipomovirus |
| NC 014791.1 Ugandan cassava brown streak virus | 58.7 | 58.9 | 58.9 | 57.9 | 62.5 | 60.3 | 56.9 | Ipomovirus |
| NC 006941.1 Cucumber vein yellowing virus | 62.7 | 60.5 | 62.7 | 60.3 | 62.7 | 60.9 | 56.7 | Ipomovirus |
| NC 003797.1 Sweet potato mild mottle virus | 61.9 | 63.3 | 60.7 | 56.9 | 61.5 | 64.5 | 59.9 | Ipomovirus |
| NC 012698.2 Cassava brown streak virus | 59.1 | 60.1 | 60.7 | 55.8 | 63.5 | 61.7 | 57.1 | Ipomovirus |
| NC 026759.1 Artichoke latent virus | 58.0 | 58.4 | 59.2 | 55.7 | 56.6 | 58.6 | 55.1 | Macluravirus |
| NC 018455.1 Chinese yam necrotic mosaic virus | 57.8 | 57.8 | 59.4 | 55.3 | 56.3 | 58.8 | 56.8 | Macluravirus |
| NC 014037.1 Sugarcane streak mosaic virus | 59.6 | 58.6 | 57.1 | 58.8 | 58.6 | 57.9 | 57.7 | Poacevirus |
| NC 012799.1 Triticum mosaic virus | 59.6 | 58.4 | 57.5 | 57.1 | 58.1 | 56.3 | 57.1 | Poacevirus |
| NC 018572.1 Caladenia virus A | 58.4 | 57.5 | 56.1 | 55.9 | 56.9 | 59.2 | 56.5 | Poacevirus |
| NC 005904.1 Hordeum mosaic virus | 68.2 | 69.6 | 64.0 | 66.0 | 69.0 | 68.2 | 62.8 | Rymovirus |
| NC 005903.1 Agropyron mosaic virus | 70.0 | 69.4 | 65.0 | 62.6 | 68.6 | 66.4 | 62.8 | Rymovirus |
| NC 001814.1 Ryegrass mosaic virus | | | | | | | | |