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# **Metagenomic analysis of virome cross-talk between cultivated *Solanum lycopersicum* and wild *Solanum nigrum***

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The assembled viral genomic reported here have been deposited in Genbank under accession numbers **MN216346** to **MN216389**. Cleaned virome sequence reads have been deposited on the INRA National Data Portal under the identifier <https://doi.org/10.15454/S486RR>.

**ABSTRACT**

Wild plants and weeds growing close to crops constitute a potential reservoir for future epidemics or for the emergence of novel viruses but the frequency and directionality of viral flow between cultivated and wild plants remains poorly documented in many cases. Here, we studied the diversity of viral populations between tomato (*Solanum lycopersicum*) and neighboring european black nightshade (*Solanum nigrum*) using high throughput sequencing (HTS) based metagenomics. A large variability in virome richness with only 17.9% shared Operational Taxonomy Units between tomato and nightshade, but this richness could not be linked to a particular host or to local conditions. A detailed population analysis based on assembled contigs for potato virus Y (PVY), broad wilt bean virus 1 and a new ilarvirus tentatively named *Solanum nigrum* ilarvirus 1 provides information on the circulation of these viruses between these two *Solanum* species and enriches our knowledge of the tomato virome.

**KEYWORDS:** metagenomics, virome, double stranded RNA (dsRNA), tomato, spillover

## INTRODUCTION

Through the past decade, metagenomics based on high throughput sequencing (HTS) has been widely used in the plant virology field, advancing our knowledge on the diversity of plant viruses. Specifically, metagenomics allowed to discover unknown viruses, explore the intraspecific genetic diversity of known viruses, and study virus ecology and epidemiology (Massart et al., 2014; Roossinck et al., 2015; Villamor et al., 2019). Plant viruses cause epidemics on all major cultures of agronomic importance, representing a serious threat to global food security. As a consequence, virologists have for a long time focused their efforts on economically important crops, often neglecting bordering weeds and wild plants (Wren et al., 2006). However, agro-ecosystems are complex environments in which crop plants sometimes interact with the in-plot and bordering weeds and wild plants. Viruses may be transferred between wild plants/weeds and crops and vice versa by a variety of mechanisms and vectors. Thus wild plants or weeds may constitute “reservoirs” of viruses that may subsequently spread to cultivated plants while crops may constitute a source from which viral infections may spillover to the wild plants/weeds compartment (Power and Mitchell, 2004).

Overall, our understanding of the details of fluxes of viruses from crops to weeds and from weeds to crops remains frequently limited. A role of a weed population as a reservoir or, alternatively, virus spillover from a crop are often assumed. Unfortunately, the techniques most frequently used for viral populations characterization, in particular serological ones, frequently do not provide sufficient intra-specific resolution to provide unambiguous conclusions on such aspects. It is then difficult to ensure that the co-occurrence of a virus in

crops and weeds reflects the transfer of isolates rather than the existence separate viral populations adapted to the two host populations.

Tomato (*Solanum lycopersicum*) is one of the most popular and extensively consumed vegetable crops. There are at least 136 characterized viral species that are capable of infecting tomato and due to global climate changes and increased international trade, the spread of known viruses to new geographic areas and the emergence of new viruses have been frequently detected in particular in recent years (Brunt, 1996; Hanssen et al., 2010). Torradoviruses (family *Secoviridae*) are an example of a group of recently emerged plant viruses, many of which affect tomato. These include for example tomato torrado virus (ToTV), which was first described from tomato in Mexico (Verbeek et al., 2008) and reported more recently in France (Verdin et al., 2009) and in other host plant species (van der Vlugt et al., 2015), as well as tomato marchitez virus (ToMarV; (Verbeek et al., 2008)) and tomato chocolàte virus (ToChV; (Verbeek et al., 2010)). Another example of recent emergence of a virus in tomato concerns tomato brown rugose fruit virus (ToBRFV), a tobamo like virus which was discovered from tomato in Israel in 2014 (Luria et al., 2017) and that has spread since then to many countries including Jordan, Mexico, the United States (Southern California), Germany, Italy, Turkey, the Netherlands and Saudi Arabia. The source(s) and cause(s) of the emergence of such novel agents is(are) frequently unknown but weed and wild plants are often considered as a major sources of future emerging viruses than may occasionally be transferred to crops (Anderson et al., 2004; Elena et al., 2014; McLeish et al., 2019).

Recently, during a study characterizing the virome of 170 field-grown tomatoes collected in China by small RNAs sequencing, Xu et al. (2017) showed that the tomato viral community is dominated by a few species, most of them being positive-sense ssRNA viruses. Multiple infections were found to be frequent as well as recombination events in viral genomes (Xu et al., 2017).

European black nightshade (*Solanum nigrum*), a wild species in the same botanical genus as tomato is a widespread weed in many countries. However, in southern India it is widely consumed and cultivated on a commercial scale (Jamuna et al., 2017) and sometimes also used as for its medicinal properties (Javed et al., 2011). *S. nigrum* is known to harbor a wide range of viruses such as begomoviruses, orthotospoviruses, potyviruses, tobamoviruses under field conditions, and has often been suspected to act as a reservoir host for viruses or for recombinant isolates infecting solanaceous crops (Holm et al., Garcia-Andr s et al., 2006; 1979; Jamuna et al., 2017).

In the present study, using a metagenomics approach, we investigated and compared the virome in tomato samples and in the related *S. nigrum* populations collected either in tomato fields or in various other environments. The comparison of these viromes provides novel insight into the viral fluxes between these two species.

## MATERIALS AND METHODS

### *Study sites and plant samples*

Virome richness and composition were analyzed in tomato (*Solanum lycopersicum*) and in European black nightshade (*Solanum nigrum*) that were growing either close to the

sampled tomato crops or in other sites, unmanaged or involving unrelated crops (sunflower, maize, sorghum and alfalfa, Table S1). In total, tomato crops were sampled in seven sites and nightshade in six of the seven tomato sites, plus in five non-tomato sites (Table S1). For each sampled plant populations, leaves from a total of 100 individual plants were collected in summer 2017 or 2018 and assembled in two pools corresponding to fifty individual plants (0.1g of leaf/plant) for nucleic acids extraction. No specific efforts were made to select symptomatic plants, but plants with obvious fungal attack, insect colonization or necrotized parts were excluded.

#### ***Double-stranded RNAs purification, library preparation and Illumina HiSeq sequencing***

Double-stranded RNAs were purified from each plant pool by two rounds of CF11 cellulose chromatography and converted to cDNA according to the protocol described by Marais et al. (2018). In parallel, a negative control blank was similarly prepared using only buffer. Whole genome amplifications (WGAs) were performed on each cDNA sample (using the same MID tag for the two pools of each sampling site), the PCR products were purified using the MinElute PCR Purification Kit (Qiagen) and their concentration determined spectrophotometrically (Marais et al., 2018). Equal quantities of the amplification products from the two pools of each sampling site were then regrouped and independent sequencing libraries prepared for each site and sequenced in multiplexed format (2×150 bp) on an Illumina HiSeq 3000 system at the GenoToul platform (INRA Toulouse, France). Cleaned virome sequence reads have been deposited on the INRA National Data Portal under the identifier <https://doi.org/10.15454/S486RR>.

***Bioinformatics analyses: Reads cleaning, contigs assembly and annotation, Operational Taxonomic Units (OTU) clustering***

Following demultiplexing, adapters and MID tags were removed with *cutadapt* (Martin, 2011), and reads were quality trimmed (minimum quality score 20, minimum length 70 nucleotides). In order to limit inter-sample cross talk associated with index-hopping (Illumina, 2017; van der Valk et al., 2019), only reads having identical MID tags on both pair members were retained for further analyses (Table S1). Contigs were *de novo* assembled for each library using IDBA-UD (<https://academic.oup.com/bioinformatics/article/28/11/1420/266973>).

All contigs were annotated using BlastN and BlastX against the NCBI Genbank non redundant nucleotide (nt) or protein databases with a conservative e-value cut-off of  $10^{-4}$ . In this way, contigs were assigned to one of the following categories: virus, eukaryote, bacteria, algae, and unknown. For viral contigs, a family-level annotation was derived from the NCBI taxonomic information for the first Blast hit.

A clustering approach (Lefebvre et al., 2019) was used to define operational taxonomy units, following the strategy highlighted by Simmonds (2015). Briefly, a search of RNA-dependent RNA polymerase (RdRp) conserved protein motifs was performed in all contigs using Reversed Position Specific Blast (RPS-Blast) (Altschul et al., 1997) against the pfam database (Bateman et al., 2018). The contigs encoding a viral RdRp motif (Table S1) were retrieved and aligned with reference sequences and distance matrices computed with the ETE3 toolkit (Huerta-Cepas et al., 2016). These matrices were used to perform a clustering allowing to regroup in a single OTU all contigs differing by less than a set



cut-off divergence value (Murtagh and Legendre, 2014). We used a 10% divergence cut-off value, because it has been shown to generate in many viral families OTUs that are a relatively good approximation of taxonomic species (Lefebvre et al., 2019). OTUs were thus defined for each RdRp family, allowing to generate an OTU table indicating for each sampling site the presence/absence and the number of reads integrated in each identified OTU (Table S1).

### ***Further viral genome assemblies, sequence comparisons and phylogenetic analyses***

When needed, contigs were extended by repeated rounds of mapping of quality-trimmed reads using CLC Genomics Workbench 11 (CLC-GWB). For some isolates/viruses, genomic scaffolds were assembled by mapping contigs and/or reads on a reference genome using CLC-GWB. Long contigs or scaffolds showing more than 75% completeness for cucumber mosaic virus (CMV), southern tomato virus (STV), broad wilt bean virus 1 (BBWV1, both genomic RNAs), the new ilarvirus (all three genomic RNAs) and potato virus Y (PVY) were used for phylogenetic analyses and have been deposited in Genbank (Accession numbers MN216356 to MN216369 (Table S2).

Multiple sequence alignments of contigs/scaffolds obtained from HTS data and of reference isolates retrieved from Genebank (or alignments of deduced encoded proteins) were performed using the ClustalW algorithm (Thompson et al., 1994) as implemented in MEGA 6.0 (Kumar et al., 2008). Phylogenetic trees were reconstructed in MEGA 6.0, using the Maximum Likelihood (ML) algorithm. Branch support was evaluated by bootstrap analysis (100 replicates).

## RESULTS

### *Comparison of the tomato and nightshade viromes at different sampling sites*

A total of 20 viral families were discovered by Blast annotation taking into account the different libraries (18 sampled plant populations) with an average of  $4.3 \pm 3.3$  families per library, but with a very large variability between the sampled plant populations. The tomato sample from the TOM3 site showed the highest number of viral families (13, Figure 1) followed by another tomato sample (TOM7, 9 viral families) and nightshade samples from the TOM2 and NIG3 sites (8 viral families). The *Potyviridae* family was represent in a total of 13 samples including both tomato (six samples) and nightshade (seven samples, of which five were from tomato sites; Figure 1). The family *Totiviridae* was represented in eight samples while at the other extreme the *Tombusviridae* family was represented in a single tomato sample from the TOM3 site. Given the high between-populations variability it was not possible to establish statistically meaningful differences in family-level richness between the tomato and nightshade populations (Figure 1).

Taking into account all sampling sites, a total of 87 unique RNA-dependent RNA polymerase (RdRp) OTUs were detected (Table S1). Similar to the family-level analysis, a very large variability was observed in the number of OTUS detected per site. The richer viromes were found in the TOM7 site tomato population and in the NIG3 nightshade population, with respectively 38 and 27 OTUs, followed by 26 OTUs for the TOM3 site tomato population. In all other samples less than 8 RdRp OTUs were detected (Table S1).

In total, 62 OTUs were identified from tomato samples and 44 from nightshade ones but this difference is largely the consequence of a single tomato sample (TOM7) which is

particularly rich in unique mycovirus-like OTUs (Table S1). Nineteen OTUs (21.8% of total) were shared between the two plant species, most of them from the families *Totiviridae*, *Partitiviridae* and *Chrysoviridae* as well as unclassified mycovirus-like OTUs. RdRP\_1-OTU\_8 which corresponds to potato virus Y was the most widely shared OTU (Table S1, see below). It explains the wide prevalence of the *Potyviridae* family described above. Twenty-five OTUs were found to be nightshade-specific, among which RdRP\_2-OTU\_13 corresponds to cucumber mosaic virus (CMV) and RdRP\_1-OTU\_14 to broad bean wilt virus 1 (BBWV1) (Table S1, see below). Forty-three OTUs were found to be tomato-specific, some of which have extremely high identity levels with known viruses such as *Sclerotinia sclerotiorum* hypovirus 1, *Sclerotinia sclerotiorum* umbra-like virus 2 or Botrytis virus F and very likely correspond to these agents (Table S1).

There were overall only very few OTUs shared between tomato and nightshade samples for a given sampling site, with PVY being the most frequent. In five sites out of six, no OTU (sites TOM2 and TOM6) or only one OTU (sites TOM1, TOM4, and TOM5) were shared, whereas in site TOM3, four OTUs were shared (Table S1, Figure S1).

### ***Near complete genome reconstruction for selected viral agents***

For several viruses, long, high quality contigs were obtained during the initial trimmed reads assembly. This concerned in particular several single-stranded RNA viruses: cucumber mosaic virus (CMV), broad wilt bean virus 1 (BBWV1, both genomic RNAs), potato virus Y (PVY), and a new ilarvirus (all three genomic RNAs) as well as a double-stranded RNA virus of the *Amalgaviridae* family, southern tomato virus (STV). In a few cases, the viral genome was unambiguously covered by a few contigs that were either

non-overlapping or had only a short overlap and which were therefore manually assembled into a scaffold by mapping contigs on a reference genome. All contigs and scaffolds were validated by visual inspection of read mappings at high stringency to ensure the absence of assembly artifacts. The corresponding sequences have been deposited in Genbank (Accession numbers MN216346 to MN216389, Table S2).

Multiple alignments and phylogenetic analyses (see below) were used to identify representative contigs for the various phylogenetic clusters of each virus. These representative contigs were in turn used as targets for the mapping of the trimmed reads of all libraries at high stringency. This allowed to evaluate the representation of each virus/variant in the virome of each sampled plant population. The low background of viral reads observed in the negative control, probably resulting from low level experimental contamination or from inter sample cross talk due to index-hopping (Illumina, 2017), was subtracted from the mapped reads numbers of each library. The results of this analysis are presented in Table S3.

CMV was detected, by high read numbers, at a single sampling site (TOM1), in the nightshade population but not in the corresponding tomato population (Table S3). All three genomic RNAs were assembled into unique long contigs of respectively 3,301 nt (RNA1; ca. 98.2% of the full length molecule), 2,996 nt (RNA2, ca. 98.3% of the full length molecule) and 2,155 nt (RNA3, ca. 97.2% of the full length molecule) but no evidence was found for presence of a CMV satellite. Despite the fact that no specific efforts were made to improve/validate the contigs further, all three genomic RNAs are extremely close to CMV sequences present in Genbank and, in particular to the I17F isolate, a subgroup I

isolate characterized from tomato in France at the beginning of the 1980's (Jacquemond and Lot, 1981). Nucleotide identity levels of respectively 99.5%, 99.4% and 99.5% for genomic RNA1, 2 and 3 (respectively 18, 18 and 11 point mutations) are thus observed between the 1981 IF17 isolate and the contigs from HTS data on a 2017 sample, highlighting both the quality of the HTS assemblies and the relative stability of the CMV population over more than 35 years.

In the case of southern tomato virus (STV), unique long contigs representing nearly complete genomes were obtained from several plant populations, representing 92.6%-99.4% of the full length genome. Coherent with the low diversity identified so far in this virus, these contigs are nearly identical to each other (<0.4% nucleotide divergence) with the exception of one contig, which diverges by 2.6%-2.8% from the others. Identity levels with isolates present in Genbank range from 100% to 95.9%, again highlighting the quality of the contigs assembled from the HTS data. Overall STV was detected in five of the seven tomato pools, an observation in accordance with the presence of this virus in a wide range of tomato varieties (Sabanadzovic et al., 2009). On the other hand, a surprising result is the detection, with higher reads number than for the tomato pools (Table S3) of STV in a nightshade pool (Nightshade-TOM5), extending the host range of this relatively recently discovered virus. The nightshade STV sequence belongs to the group of closely related isolates and does not present obvious specific molecular properties (data not shown).

#### ***Broad bean wilt virus 1 populations diversity***

Broad bean wilt virus 1 (BBWV1) was detected in six of the sampled nightshade populations (out of a total of 11 populations, 55%) and was not detected in any of the

sampld tomato populations (seven populations, Table S3). The assembly of the BBWV1 reads from the various nightshade populations highlighted a complex viral population structure with a total of five RNA1 clusters and three RNA2 clusters identified (Table S3, see below). On average, the reconstructed genomic sequences represented 94.3%  $\pm$  3.9% of the BBWV1 RNA1 (87.8%-99.3%, depending on the contigs) and 87.8%  $\pm$  11.9% of the BBWV1 RNA2 (73.4%-96.6%). For one sample, it was not possible to reconstruct more than 60% of the RNA2 sequence and the corresponding scaffold was therefore not included in further analyses. The average nucleotide divergence between the RNA1 clusters, calculated on representative isolates is 16.6%  $\pm$  0.3% (13.4%-17.6%), explaining the effective separate assembly in cases of mixed infection by isolates belonging to different clusters. For the three RNA2 clusters, the corresponding divergence values are 15.8%  $\pm$  0.6% (10.5%-18.6%). Mapping of reads at high stringency on contigs representative of the various clusters allowed to describe the BBWV1 population present in the various plant populations. Isolates representative of between one and four RNA1 clusters and of one or two RNA2 clusters could thus be detected at individual sampling sites, with some sites providing evidence of only a single RNA1-RNA2 combination, while at the other extreme, one site showed the presence of four RNA1 clusters and a single RNA2 one. Another site showed the presence of a single RNA1 cluster but of two RNA2 ones (Table S3). Taken together, these elements suggest the frequent occurrence of reassortment between BBWV1 genomic segments in the sampled nightshade populations.

Phylogenetic analyses performed on the RNA1 and RNA2 sequences derived from the HTS data and from all full length isolates present in Genbank (Figure 2A and 2B)

demonstrate that the BBWV1 isolates present in the nightshade populations sampled here largely expand the known BBWV1 diversity. Indeed, the HTS-derived sequences cluster separately from reference full-length sequences available to date and are, on average, highly divergent from them with an average intergroup distance of 17.0% +/- 0.4% for RNA1 and 19.0% +/- 0.6% for RNA2.

#### ***Presence of a novel ilarvirus in the sampled nightshade and tomato populations***

Long, high quality contigs representative of an ilarvirus were identified in several libraries. The contigs corresponding to the three genomic RNAs were further extended and validated for the NIG4 sampling site, allowing to reconstruct near complete molecules. Indeed, a comparison with the genomic RNAs of Parietaria mottle virus (PMoV), the closest characterized ilarvirus (see below) indicated that all five open reading frames (ORFs) [coding respectively for P1 (RNA1), P2 and P2b (RNA2) and the movement (MP) and coat proteins (CP) (RNA3)] were complete, with the exception of ORF2 which misses an estimated 62 nt (21 N-terminal amino acids missing from the P2 protein sequence). The contigs are respectively 3,445, 2,757 and 2,257 nt long for RNA1, RNA2 and RNA3, representing respectively 97.9%, 94.4% and 100.5% of the length of the corresponding genomic RNAs of the reference PMoV isolate (NC\_005848, -49 and -54). These genomic sequences have been deposited in Genbank under Accession numbers MN216370 to MN216378. Blast analyses indicated that the virus is most closely related to PMoV and to several other subgroup 1 ilarviruses and this proximity was confirmed by phylogenetic analyses performed on all genome encoded proteins (Figure 3A and 3B, Figure S2). However, these phylogenetic trees demonstrate that the virus is not substantially more

related to PMoV than to any other approved species in that small ensemble. The significant divergence of the virus from PMoV is confirmed by sequence comparisons, the deduced proteins being only 81.8% (P1) to 53.9% (CP) identical with those of PMoV while the genomic RNAs show only 73.2% (RNA1) to 58.6% (RNA3) nucleotide identity (Table S4). Taken together these results suggest that the detected larvirus is a new subgroup 1 member for which the name *Solanum nigrum* ilarvirus 1 (SnIV1) is proposed.

Mapping of the reads from each plant population on the SnIV1 genomic RNAs showed that this virus was present in eight of them, corresponding to 6/11 nightshade populations (54.5%) and, represented by relatively low read numbers, to 2/7 tomato populations (28%) (Table S3).

#### ***Analysis of PVY populations in the sampled nightshade and tomato populations***

As for the other viruses, long, high quality contigs were obtained in most cases for PVY. In a few cases, probably resulting from low reads numbers or from the simultaneous presence of closely related isolates in the sampled plant populations, only short PVY contigs were obtained for some isolates. However, from all plants populations with high PVY read numbers, one to three long contigs could be assembled presenting on average 95.4% +/- 4.8% of the full length PVY genome (85.3%-99.9%). A phylogenetic analysis of these contigs, together with representative reference isolates retrieved from Genbank (Figure 4) shows a very contrasted situation, with on the one hand, a large number of sequences forming a very tight cluster corresponding to PVY-NTN and, on the other, a much more diverse second cluster corresponding to PVY-C. No isolates representative of the PVY-O and PVY-N strains were observed nor some of their frequent recombinants such as PVY-Wilga



(Figure 4). In total, 10 contigs were obtained for PVY-NTN (five from tomato and five from nightshade) and four for PVY-C (three from tomato and one from nightshade).

The reads from all plant populations were then mapped on selected contigs representative of PVY-NTN and of the three PVY-C variants identified, using stringent parameters so as to limit cross-mapping between isolates. Under these conditions, from one to four PVY variants could be detected in the analyzed plant populations. Some populations showed extremely low read numbers (<90), which is suggestive of an absence or a very low prevalence of PVY in the corresponding plant populations. Remarkably, this situation corresponds to 2/7 (28.6%) tomato populations, to 2/6 (33.3%) nightshade populations growing side by side with tomato but to 4/5 (80%) of the nightshade population growing away from tomato.

As judged from the mapping results, the two most frequent PVY strains were PVY-NTN, which was detected in all tomato and nightshade populations in which PVY was detected, and isolates with mapping affinities with isolate TOM7-C, which clusters together with the French PVY-C1 SON41 pepper isolate (Table S3 and Figure 4). By contrast, isolates corresponding to the two other PVY-C mapping references used were only detected in one to three of the sampled plant populations. The frequency of detection of the various clades does not seem to differ much between tomato and nightshade (Table S3).

## DISCUSSION

The viromes characterized in the present work vary greatly between the sampled plant populations and, for some of them, showed only a limited number of OTUs or of viral

families despite the size of the composite plant samples analyzed. This might reflect the impact of fungicide treatments in the sampled crops which might have reduced fungal diversity and in turn the ability to detect mycoviral communities associated with the sampled plants. It should however be stressed that the OTU-based analysis provides a lower bound estimate of viral diversity since viruses for which the genome region encoding the conserved RdRp motif is not represented in the assembled contigs will not be identified by a corresponding OTU. On the other hand, competition between the sequence of different viruses for representation in the sequencing reads is unlikely to have adversely impacted the richness of the identified viromes since the three richest viromes were identified in plant populations for which the percentage of mapped viral reads was not obviously higher (or lower) than that observed in samples with a much lower viral diversity (Tables S1 and S2).

In contrast to a recent virome study of 170 tomato samples which indicated that diverse ssRNA viruses represented 77% of the identified viruses (Xu et al., 2017), they represented only 12.6% of the viral OTUs identified here (Table S1). The corresponding value for dsRNA viruses is 26.4% while unassigned or unannotated agents accounted for a cumulated 60.9%. Whether this difference is a consequence of differences in the methodology used or actually reflects differences in the analyzed viromes cannot easily be ascertained. However, some frequent viruses of tomato such as PVY, CMV or STV were detected in both studies (Xu et al., 2017).

Despite the use of complex plant pools composed of 100 individual plants, we were able to assemble long, high quality contigs for some viruses (PVY, BBWV1, STV, and the new

SnIV1), covering a very high proportion of the genome of these agents. In a few cases, such long contigs could not be assembled, possibly as a consequence of too low coverage and read numbers, or because mixed infection involving closely related variants created problems during contig assembly. Indeed, there is some evidence that at least one additional clade of PVY existed in some tomato samples as judged by the detection of some partial contigs diverging from the fully assembled genomes (data not shown).

For read mapping, stringent parameters were used so that there is no or extremely limited cross talk between isolates of different clades, as seen by reads numbers in the case of BBWV1 and PVY (Table S2). At the same time, it is difficult to know precisely how to interpret the samples with a very low number of reads mapped. Even if the background observed in the negative control was subtracted, this cross-talk background likely due to index hopping (Illumina, 2017; van der Valk et al., 2019) may not be completely uniform from sample to sample. These low read numbers may therefore either reflect an absence of the virus but a low, slightly uneven cross-talk with other samples or a true, very low prevalence of the virus in the sampled population. It is not possible to decide between these two options here.

A very large and unexpected BBWV1 diversity was identified in the sampled nightshade populations. The analysis of BBWV1 populations suggests the existence of frequent reassortment between RNA1 and RNA2 variants, an observation in line with the results of (Ferriol et al., 2014). BBWV1 is a Fabavirus with a relatively wide host range and which is pathogenic on a range of crops including broad bean, pea, spinach, lettuce, pepper and, occasionally, tomato (Blancard, 2012; Carpino et al., 2019; Taylor and Stubbs, 1972). It is

therefore surprising that this aphid-transmitted virus was only detected from nightshade samples in this study. This observation suggest the existence of a biological or epidemiological barrier limiting the spread of BBWV1 from nightshade to tomato. In this respect, it is noteworthy that during a recent comparison of BBWV1 isolates, infection rates in tomato following artificial inoculations ranged only from 40% to 60% for four genetically different BBWV1 isolates (Carpino et al., 2019).

The novel ilarvirus here named *Solanum nigrum* ilarvirus 1 (SnIV1) was detected in both tomato and nightshade samples. However, both the prevalence and, with one exception, the read numbers of SnIV1 appear to be higher in the nightshade populations than in the tomato ones. On the other hand, the presence of SnIV1 in nightshade samples does not seem to be affected by whether they were growing side by side with tomato or not (respectively 3/6 and 3/5 cases, Table S2). Interestingly, reanalysis of metagenomics data showed that this virus was already present in 2011 at the TOM3 site, in *S. villosum* (hairy nightshade) a close relative of *S. nigrum*. Whether this novel ilarvirus is pathogenic to tomato or whether it has the potential to emerge at some point as a tomato pathogen in the same fashion as its close relatives *Parietaria* mottle virus (Roggero et al., 2000) and tomato necrotic spot virus (Batuman et al., 2011) remains to be evaluated.

The main PVY strains identified in this study were PVY-NTN and PVY-C1. PVY-C1 isolates were mainly detected from tomato, with one isolate shared between tomato and nightshade in the TOM3 site (Figure 4). On the other hand, PVY-NTN isolates were found in both tomato (5/7 samples) and nightshade samples (6/11 samples) from a total of seven of the 12 sampling sites. Interestingly, PVY populations at the TOM3 site had been studied

2011-2012 using specific RT-PCR assays (Moury et al., 2017). At the time, PVY-C1 and recombinant isolates likely to represent PVY-NTN were detected in tomato, while a more diverse population involving PVY-O, PVY-NTN, PVY-N and PVY-C1 was detected in nightshade and in the related *S. villosum* (Moury et al., 2017). The results reported here therefore suggest a simplification of the PVY nightshade population at that site, with the loss of PVY-O and PVY-N, possibly as a consequence of the competition with PVY-NTN and C1.

A noteworthy observation concerns PVY prevalence in nightshade populations at tomato sites (4/6 sites, 66.6%) and at non-tomato sites (1/5 sites, 20%). This suggests that infection in nightshade is greatly increased by the presence of tomato, reflecting a likely spillover effect from tomato crops to the wild nightshade population (Power and Mitchell, 2004).

Taken together the results reported here provide evidence for viral exchanges between tomato and nightshade populations growing side by side (such as the extremely closely related tomato and nightshade PVY isolates shared at the TOM3 site or the low detection of the new ilarvirus in tomato only at sites where it is also present in nightshade). At the same time, our results also highlight situations where an expected transfer is not observed, likely as a consequence of unforeseen biological or ecological barriers. This concerns in particular BBWV1 only found in nightshade when there are numerous indications that this virus should be able to infect tomato (Carpino et al., 2019). These results also highlight the power of metagenomics to analyze viral exchanges in complex plant populations, from the overall virome structure down to the intra-specific variability level, revealing unknown novel agents but also unforeseen biological processes.

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## REFERENCES

- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389-3402.
- Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., Daszak, P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol. Evol.* 19, 535-544.
- Bateman, A., Smart, A., Luciani, A., Salazar, G.A., Mistry, J., Richardson, L.J., Qureshi, M., El-Gebali, S., Potter, S.C., Finn, R.D., Eddy, S.R., Sonnhammer, E.L.L., Piovesan, D., Paladin, L., Tosatto, S.C.E., Hirsh, L., 2018. The Pfam protein families database in 2019. *Nucleic Acids Res.* 47, D427-D432.
- Batuman, O., Chen, L., Gilbertson, R., 2011. Characterization of Tomato necrotic spot virus (ToNSV), a new ilarvirus species infecting processing tomatoes in the Central Valley of California, *Phytopathology* 103, 1391-1396.
- Blancard, D., 2012. Tomato diseases: identification, biology and control: A Colour Handbook. CRC Press, USA.
- Brunt, A., 1996. Plant Viruses Online: Descriptions and Lists from the VIDE Database. Ver. 20. <http://biology.anu.edu.au/Groups/MES/vide/>.
- Carpino, C., Elvira-González, L., Rubio, L., Peri, E., Davino, S., Galipienso, L., 2019. A comparative study of viral infectivity, accumulation and symptoms induced by broad bean wilt virus 1 isolates. *J. Plant Pathol.* 101, 275-281.
- Elena, S.F., Fraile, A., Garcia-Arenal, F., 2014. Evolution and emergence of plant viruses. *Adv. Virus Res.* 88, 161-191.
- Ferriol, I., Ferrer, R.M., Luis-Arteaga, M., Guerri, J., Moreno, P., Rubio, L., 2014. Genetic variability and evolution of broad bean wilt virus 1: role of recombination, selection and gene flow. *Arch. Virol.* 159, 779-784.

- García-Andrés, S., Monci, F., Navas-Castillo, J., Moriones, E., 2006. Begomovirus genetic diversity in the native plant reservoir *Solanum nigrum*: evidence for the presence of a new virus species of recombinant nature. *Virology* 350, 433-442.
- Hanssen, I.M., Lapidot, M., Thomma, B.P., 2010. Emerging viral diseases of tomato crops. *Mol. Plant. Microbe Interact.* 23, 539-548.
- Holm, L., Pancho, J.V., Herberger, J.P., Plucknett, D.L., 1979. A geographical atlas of world weeds. John Wiley and Sons, New York, USA.
- Huerta-Cepas, J., Bork, P., Serra, F., 2016. ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. *Mol. Biol. Evol.* 33, 1635-1638.
- Illumina, 2017. Effects of index misassignment on multiplexing and downstream analysis. <https://www.illumina.com/content/dam/illumina-marketing/documents/products/whitepapers/index-hopping-white-paper-770-2017-004.pdf>.
- Jacquemond, M., Lot, H., 1981. L'ARN satellite du virus de la mosaïque du concombre I. - Comparaison de l'aptitude à induire la nécrose de la tomate d'ARN satellites isolés de plusieurs souches du virus. *Agronomie* 1, 927-932.
- Jamuna, S., Rajendran, L., Haokip, B.D., Nagendran, K., Karthikeyan, G., Manoranjitham, S.K., 2017. First Report of Natural Infection of *Solanum nigrum* with Tomato mosaic virus in India. *Plant Dis.* 102, 1044-1044.
- Javed, T., Ashfaq, U.A., Riaz, S., Rehman, S., Riazuddin, S., 2011. In-vitro antiviral activity of *Solanum nigrum* against Hepatitis C Virus. *Virol J.* 8, 26-26.
- Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9, 299-306.



- 465 Lefebvre, M., Theil, S., Ma, Y., Candresse, T., 2019. The VirAnnot pipeline: a resource for  
466 automated viral diversity estimation and operational taxonomy units (OTU)  
467 assignation for virome sequencing data. *Phytobiomes Journal*, online first,  
468 DOI:10.1094/PBIOMES-07-19-0037-A.
- 469 Luria, N., Smith, E., Reingold, V., Bekelman, I., Lapidot, M., Levin, I., Elad, N., Tam, Y.,  
470 Sela, N., Abu-Ras, A., 2017. A new Israeli Tobamovirus isolate infects tomato plants  
471 harboring Tm-22 resistance genes. *PLoS One* 12, e0170429.
- 472 Marais, A., Faure, C., Bergey, B., Candresse, T., 2018. Viral Double-Stranded RNAs  
473 (dsRNAs) from Plants: Alternative Nucleic Acid Substrates for High-Throughput  
474 Sequencing. *Methods Mol. Biol.* 1746, 45-53.
- 475 Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing  
476 reads. *EMBnet J* 17, 10-12.
- 477 Massart, S., Olmos, A., Jijakli, H., Candresse, T., 2014. Current impact and future  
478 directions of high throughput sequencing in plant virus diagnostics. *Virus Res.* 188,  
479 90-96.
- 480 McLeish, M.J., Fraile, A., García-Arenal, F., 2019. Evolution of plant–virus interactions:  
481 host range and virus emergence. *Curr. Opin. Virol.* 34, 50-55.
- 482 Moury, B., Simon, V., Faure, C., Svanella-Dumas, L., Marais, A., Candresse, T., 2017. Host  
483 groups of Potato virus Y: Vanishing barriers. In *Potato virus Y: biodiversity,*  
484 *pathogenicity, epidemiology and management.* C. Lacomme, L. Glais, D.U. Bellstedt,  
485 B. Dupuis, A.V. Karasev & E. Jacquot, Eds. dir., *Potato virus Y: biodiversity,*  
486 *pathogenicity, epidemiology and management* (p. 243-261). Springer, Cham. pp.  
487 243-261.
- 488 Murtagh, F., Legendre, P., 2014. Ward’s Hierarchical Agglomerative Clustering Method:  
489 Which Algorithms Implement Ward’s Criterion? *J Classif* 31, 274-295.
- 490 Power, Alison G., Mitchell, Charles E., 2004. Pathogen Spillover in Disease Epidemics.  
491 *The American Naturalist* 164, S79-S89.

492

493 Roggero, P., Ciuffo, M., Katis, N., Alioto, D., Crescenzi, A., Parrella, G., Gallitelli, D.,  
494 2000. Necrotic disease in tomatoes in Greece and southern Italy caused by the tomato  
495 strain of Parietaria mottle virus. *J. Plant Pathol.* 82, 159.

496 Roossinck, M.J., Martin, D.P., Roumagnac, P., 2015. Plant Virus Metagenomics: Advances  
497 in Virus Discovery. *Phytopathology* 105, 716-727.

498 Sabanadzovic, S., Valverde, R.A., Brown, J.K., Martin, R.R., Tzanetakis, I.E., 2009.  
499 Southern tomato virus: The link between the families Totiviridae and Partitiviridae.  
500 *Virus Res.* 140, 130-137.

501 Simmonds, P., 2015. Methods for virus classification and the challenge of incorporating  
502 metagenomic sequence data. *J Gen Virol* 96, 1193-1206.

503 Taylor, R.H., Stubbs, L.L., 1972. Broad bean wilt virus 1.CMI-AAB Description of plant  
504 viruses, 81, <http://www.dpvweb.net/dpv/showadpv.php?dpvno=81>

505 Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the  
506 sensitivity of progressive multiple sequence alignment through sequence weighting,  
507 position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22,  
508 4673-4680.

509 van der Valk, T., Vezzi, F., Ormestad, M., Dalén, L., Guschanski, K., 2019. Index hopping  
510 on the Illumina HiseqX platform and its consequences for ancient DNA studies. *Mol.*  
511 *Ecol. Resour.* 2019; 00:1-11. <https://doi.org/10.1111/1755-0998.13009>.

512 van der Vlugt, R.A., Verbeek, M., Dullemans, A.M., Wintermantel, W.M., Cuellar, W.J.,  
513 Fox, A., Thompson, J.R., 2015. Torradoviruses. *Annu. Rev. Phytopathol.* 53,  
514 485-512.

515 Verbeek, M., Dullemans, A., van den Heuvel, H., Maris, P., van der Vlugt, R., 2010.  
516 Tomato chocolate virus: a new plant virus infecting tomato and a proposed member  
517 of the genus Torradovirus. *Arch. Virol.* 155, 751-755.

518 Verbeek, M., Dullemans, A.M., van den Heuvel, J.F., Maris, P.C., van der Vlugt, R.A.,

519 2008. Tomato marchitez virus, a new plant picorna-like virus from tomato related to  
520 tomato torrado virus. Arch. Virol. 153, 127-134.  
521

- 522 Verdin, E., Gognalons, P., Wipf-Scheibel, C., Bornard, I., Ridray, G., Schoen, L., Lecoq, H.,  
 523 2009. First report of Tomato torrado virus in tomato crops in France. *Plant Dis.* 93,  
 524 1352-1352.
- 525 Villamor, D.E.V., Ho, T., Al Rwahnih, M., Martin, R.R., Tzanetakis, I.E., 2019. High  
 526 Throughput Sequencing For Plant Virus Detection and Discovery. *Phytopathology*  
 527 109, 716-725.
- 528 Wren, J.D., Roossinck, M.J., Nelson, R.S., Scheets, K., Palmer, M.W., Melcher, U., 2006.  
 529 Plant Virus Biodiversity and Ecology. *PLoS Biol.* 4, e80.
- 530 Xu, C., Sun, X., Taylor, A., Jiao, C., Xu, Y., Cai, X., Wang, X., Ge, C., Pan, G., Wang, Q.,  
 531 Fei, Z., Wang, Q., 2017. Diversity, Distribution, and Evolution of Tomato Viruses in  
 532 China Uncovered by Small RNA Sequencing. *J. Virol.* 91, e00173-00117.

## LEGENDS TO THE FIGURES

**Figure 1. Barplot illustrating the presence/absence data based on Blast annotation for identified viral families in each sampled plant population.**

**Figure 2. Maximum Likelihood trees reconstructed from the alignment of near complete nucleotide sequences of RNA1 (A) and RNA2 (B) of broad bean wilt virus 1 (BBWV1) isolates and other *Fabavirus* members.** Statistical significance of the branches was evaluated by bootstrap analysis (100 replicates) and only bootstrap values higher than 70% are indicated. The scale bars represent 0.1 substitutions per site. Sequences of BBWV1 determined in this work are indicated by a black diamond. The abbreviations followed by the accession numbers are: BBWV2: broad bean wilt virus 2; GeMV: gentian mosaic virus; LLMV: Lamium mild mosaic virus; PeLaV: peach leaf pitting-associated virus; PrVF: Prunus virus F; ChVF: cherry virus F; GFabV: grapevine fabavirus.

**Figure 3. Maximum Likelihood trees reconstructed from the alignment of amino acid sequences of the P1 protein (A) and coat protein (B) of representative members of the genus *Ilarvirus*.** Statistical significance of branches was evaluated by bootstrap analysis (100 replicates) and only bootstrap values higher than 70% are indicated. The scale bars represent 0.2 substitutions per site. *Solanum nigrum* ilarvirus 1 (SnIV1) characterized in this study is indicated by a black diamond.

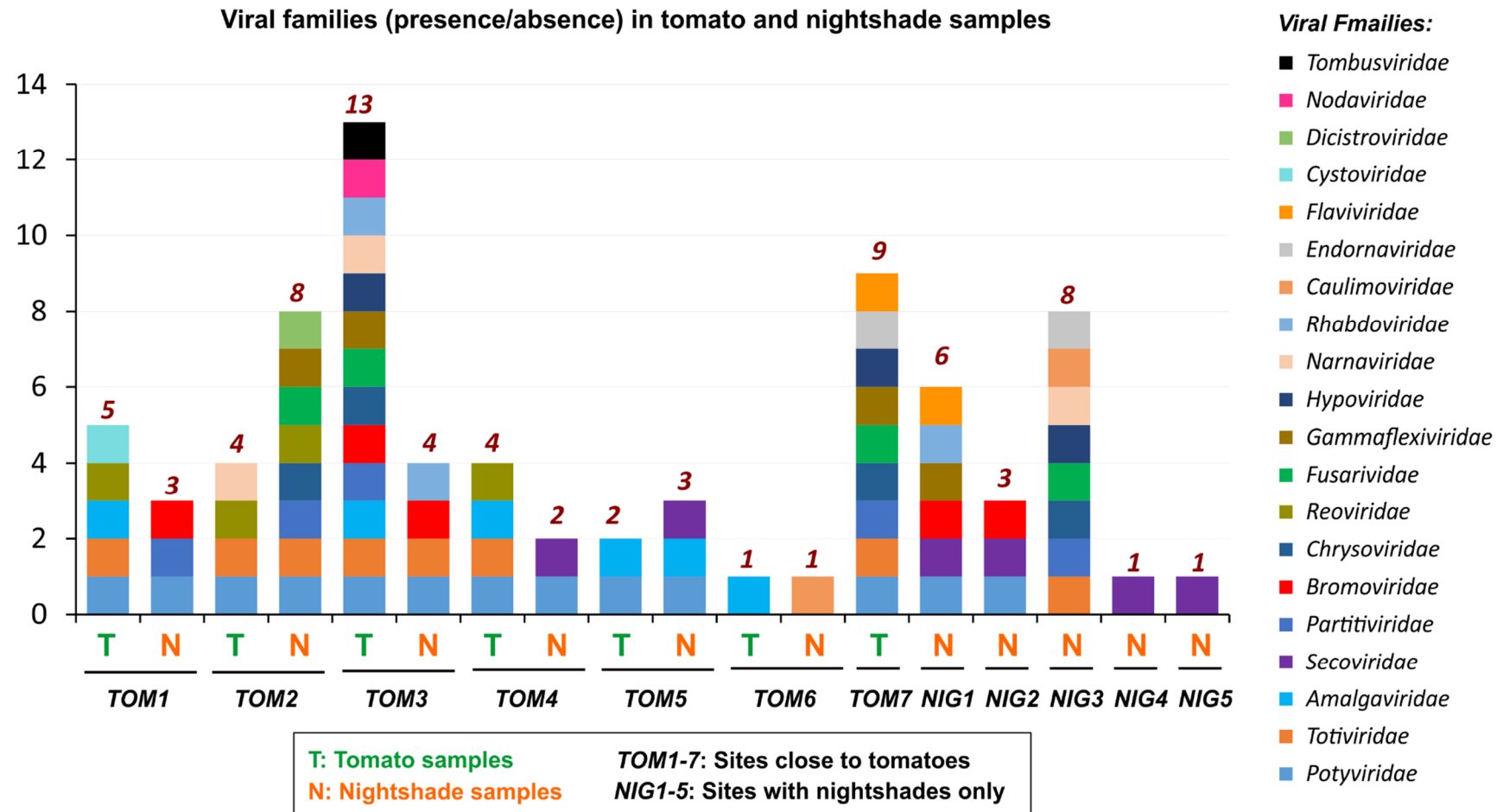
**Figure 4. Maximum Likelihood tree of the near complete nucleotide genome sequences of potato virus Y (PVY) isolates determined in this study (indicated by diamonds) and reference sequences.** PVY isolates from tomato samples are indicated by red diamonds and those from nightshade samples by blue diamonds. The tree was constructed by the Maximum Likelihood method and the statistical significance of branches was evaluated by bootstrap analysis (100 replicates). Only bootstrap values higher than 70% are indicated. The scale bar represents 0.05 substitutions per site.

**Legends to Supplementary Figures**

**Figure S1. Between samples virome cross-talk at OTU level.** The sample/library and identified number of OTUs are indicated at bottom-left; the interactions between different viromes were shown in the matrix layout at the bottom-right, the aggregates based on the groupings and the corresponding numbers of OTUs were plotted and shown in the upper part.

**Figure S2. Maximum Likelyhood trees reconstructed from the alignment of amino acid sequences of the P2a, P2b and movement (MP) proteins of representative members of the genus *Ilarvirus*.** Statistical significance of branches was evaluated by bootstrap analysis (100 replicates). Only bootstrap values higher than 70% are indicated. The scale bars represent 0.2 substitutions per site. *Solanum nigrum* ilarvirus 1 (SnIV1) characterized in this study is indicated by a black diamond.

571 **Figure 1**

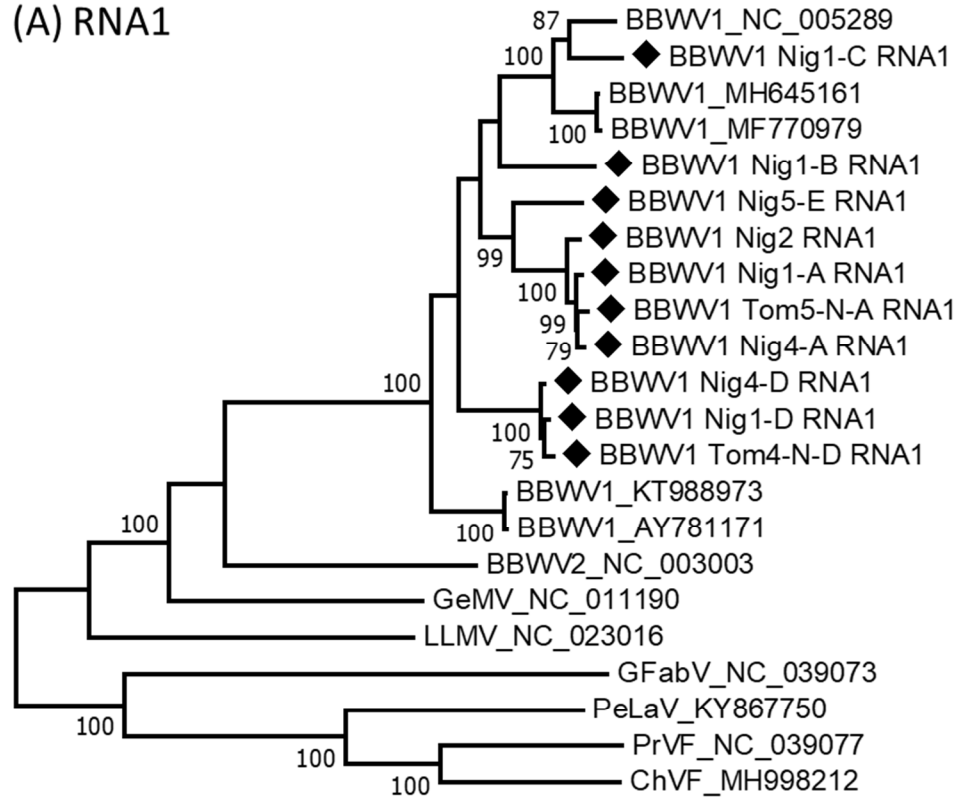


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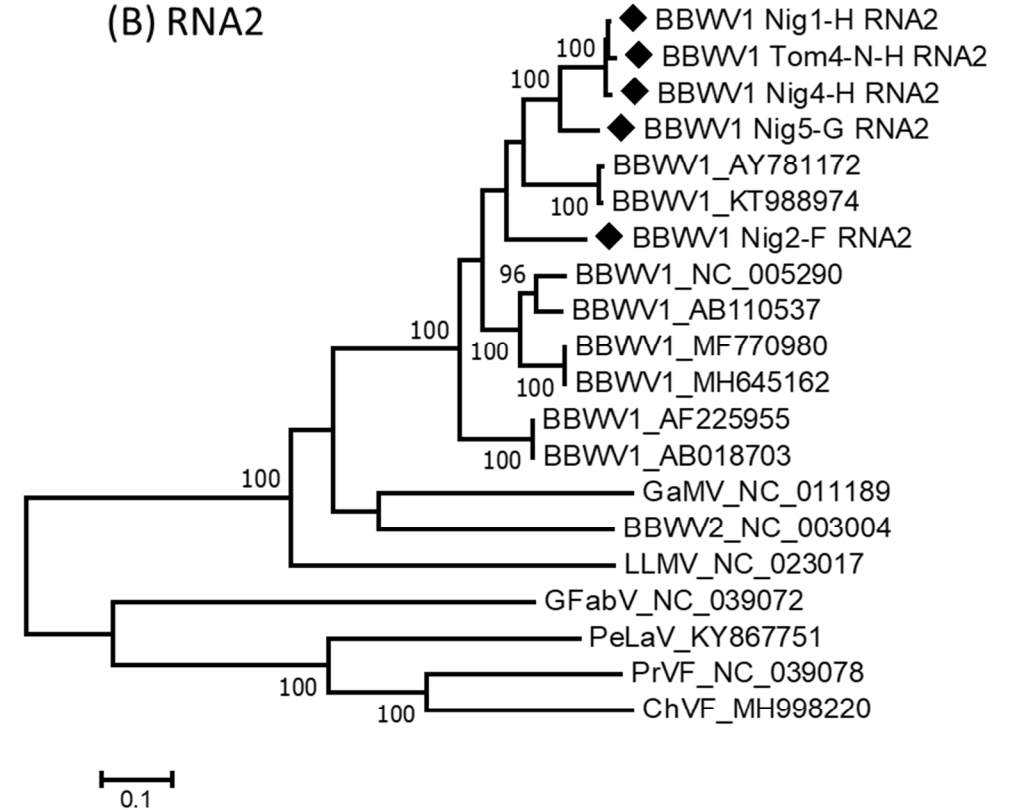
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574 **Figure 2.**

(A) RNA1



(B) RNA2

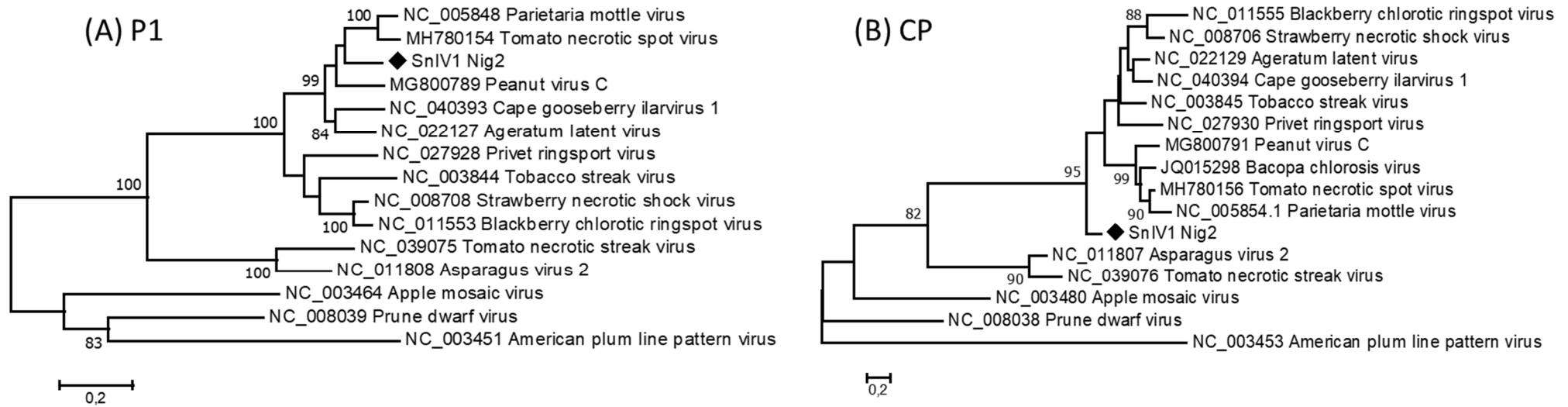


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577 **Figure 3.**



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**Figure 4.**

