



Potato Virus Y and Shallot Latent Virus of Kajoran Horticultural Production Center, Magelang Regency, Indonesia: Molecular Characterization Case Study

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Abstract

Kajoran Sub-district is a horticultural production center that supplies horticultural commodities for Central Java Province, especially Magelang. However, plant viral diseases of Kajoran have not been studied. This paper aimed to identify plant viral diseases in this horticultural production center. Some samples were taken: three potatoes (*Solanum tuberosum*) and three shallots (*Allium cepa* var. *aggregatum*), then tested respectively with RT-PCR, using two pairs of universal primers to detect *Potyvirus* and *Carlavirus*. The result showed two potato samples were infected by *Potyvirus* (potato virus Y/PVY) and three shallot samples by *Carlavirus* (shallot latent virus/SLV). GenBank accession no. OR180052 to OR180056 were assigned to the obtained partial sequences of the five isolates. The phylogenetic analysis makes the two new PVY Indonesian isolates in strain N group thus confirmed the presence of the first PVY^N in Indonesia. The constructed SLV phylogenetic tree clusters the new three isolates with eight shallot isolates in a group and separated it from isolates from other plant species. The tested PVY isolates shared 95.2 to 100% nucleotide identities among them, while 78.9 to 98.5% identities were estimated among compared SLV isolates. This study expands our understanding of the genetic variation of PVY and SLV in Indonesia, potentially leading us to find the perfect management for the viruses.

Keywords: genetic diversity; identity percentage; molecular detection; phylogenetic tree; phylogroup

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INTRODUCTION

Potato (*Solanum tuberosum*) is one of main sources of carbohydrates in several countries, including Indonesia (Nurbudiati and Wulandari, 2020). Meanwhile, shallot (*Allium cepa* var. *aggregatum*) is a valuable horticultural product that Indonesians use as the main ingredient of their traditional cooking. Unfortunately, the cultivation of both commodities is under constant threat from diseases caused by viruses belong to the genera *Potyvirus* and *Carlavirus* which may

significantly reduce the yields. Members of *Potyvirus*: potato virus Y (PVY) (Damayanti et al., 2014) and *Carlavirus*: potato virus S (PVS) (Topkaya et al., 2023) are found infecting potatoes. Meanwhile, *Potyvirus*: onion yellow dwarf virus (OYDV) and leek yellow stripe virus (LYSV), and *Carlavirus*: shallot latent virus (SLV) and garlic common latent virus (GCLV) are reported frequently found in shallot and onion (Pauzi et al., 2018; Santosa and Ertunc, 2020).

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Potyvirus is a group of plant viruses that elongated thread-like particles around 800 nm in length and 10 to 20 nm in diameter (Mahmoud et al., 2007). Genome of *Potyvirus* is single-stranded RNA (ssRNA) with one open reading frame (ORF) encoding a long ‘polyprotein precursor’ which is split into ten short proteins, including coat protein (CP) (Verma et al., 2015; Gupta et al., 2017). The shape of *Carlavirus* virion is also elongated and flexuous with a length of around 500 nm and a diameter of 12 to 13 nm. Its ssRNA genome consists of six ORFs, the CP is encoded by ORF5 (Wang et al., 2018; Li et al., 2023). In the absence of complete genome sequence, the phylogeny of *Potyvirus* and *Carlavirus* usually can be constructed using CP sequences, which is the most conserved aspect in the respective genomes (Gibbs and Ohshima, 2010; Santosa and Ertunc, 2021).

Mosaic and mottle are the typical symptoms of PVY in potatoes. However, infection in the early stage of potato plant can lead to severe leaf crinkle, chlorosis and even stunting (Gray et al., 2010). The virus is divided into O, C and Z strains according to interaction with three potato genes: *Ny*, *Nc* and *Nz*, respectively, which resulted in hypersensitive resistance (HR) response. The N and E strains do not show HR response to any of the three genes, but N produces vein necrosis, meanwhile the E strain induce mosaic and vein clearing on infected tobacco (Green et al., 2017). The latest fact in Europe, in the agricultural sector, there is a production derivation which is estimated at 187 million EUR per year. The 96 million loss comes from seed; meanwhile, 91 million comes from tuber production. Most of the derivation is not only caused by weight reduction but also caused by chemical application cost on seeds. Proper use of pesticides, perfect timing and dosage have to be perfectly arranged for cost efficiency (Dupuis et al., 2023).

PVY in potatoes was observed for the first time in Indonesia by Brawijaya University using mechanical transmission and serological (micropicipitin) tests (Mufidah et al., 2008). The virus was molecularly detected in potatoes in Lembang Sub-district, West Java Province (Damayanti et al., 2014), Karo Regency, North Sumatera Province (Mastura et al., 2018) and Wonosobo and Banjarnegara Regencies, Central Java Province (Fitriyati et al., 2019). There has not been much research concern on molecular data of PVY distribution and genetic variation in Indonesia, so it has to be enriched.

Carlaviruses (SLV and GCLV) are usually observed to be non-symptomatic in shallot, onion (*Allium cepa*) and garlic (*Allium sativum*). However, it potentially causes high yield losses on garlic when together with potyviruses (OYDV and LYSV) synergistically forming a “garlic viral complex” (Fajardo et al., 2001). Research on the spreading and genetic diversity of OYDV, LYSV, SLV and GCLV in *Allium* spp. in Indonesia also needs to be enriched (Nurviani et al., 2017; Nurenik et al., 2021; Hidayat et al., 2023).

Kajoran Sub-district is a highland, precisely at the mountain pass of Mount Sumbing, Central Java. Geographical condition of Kajoran makes it a suitable location for cultivation center of diverse horticultural crops. A sustainable and environment-friendly approach that minimizes either unnecessary or wrong pesticide application can be developed based on the accurate identification of plant diseases, especially at the early stage of planting (Mahlein, 2016; Kolagani et al., 2023). However, presence of plant viruses in Kajoran has never been surveyed. This research aims to collect samples and test them molecularly using RT-PCR as the first attempt to detect species of *Potyvirus* and *Carlavirus* that may affect potato and shallot of Kajoran. The obtained novel molecular data can enrich our knowledge of the diversity of plant viruses in Indonesia which is useful for further research.

MATERIALS AND METHOD

Samples collection

Three symptomatic potato and onion samples were collected during a field trip in Kajoran, Magelang Regency, on 18 March 2023. The samples were brought to Plant Virology Laboratory of Universitas Gadjah Mada to be kept at 4 °C until further use.

Molecular detection

Total RNA from the samples was extracted using ‘Total RNA Mini Kit’ (Geneaid Biotech Ltd., Taiwan) according to the kit standard procedure. cDNA synthesis was carried out using ReverTra Ace kit (Toyobo, Japan) in a reaction volume of 10 µl: 2 µl RNA, 2 µl 5x RT Buffer, 1 µl dNTP, 0.5 µl (10 pmol µl⁻¹) Poty 1 primer (5'-GGATCCCGGGTTTTTTTTTTTTTTTTTTT-3') (Gibbs and Mackenzie, 1997), 0.5 µl RNase inhibitor, 0.5 µl ReverTraAce® and 3.5 µl nuclease-free water. The reverse transcription program was 42 °C for 20 minutes, followed by 99 °C for 5 minutes.

The obtained cDNAs were used as templates in the subsequent PCRs, each in a reaction volume of 10 μ l: 5 μ l of MyTaq HS Red Mix (Bioline, Germany), 0.5 μ l (10 pmol μ l⁻¹) each of Poty 1 primer as the reverse primer and U341 primer (5'-CCGGAATTCATGRTITGGTGYATIGAIAAYGG-3') as the forward primer for amplification of 600 to 800 bp of 3'-end of genome of potyviruses (Langeveld et al., 1991) or AlcarF primer (5'-TGCTGCYTTTGATACYTTCGAT-3') as the forward primer for amplification of 715 bp of 3'-end of genome of carlaviruses (Cremer et al., 2021), 1 μ l of cDNA and 3 μ l of PCR-grade water. The thermal cycler program was 95 °C for 3 minutes as a pre-denaturation, 35 cycles of 95 °C for 1 minute, 56 °C for 1 minute and 72 °C for 1 minute, followed by 72 °C for 10 minutes as a final extension.

Specific target band on the agarose gel stained with Ethidium bromide was observed using a UV transilluminator (Optima Inc., Japan) after getting electrophoresis for 50 minutes at 100 V. The successfully amplified PCR products were delivered to a biotechnology company (1st BASE, Malaysia) for Bi-directional Sanger Sequencing. The obtained nucleotide sequences were run in nucleotide BLAST (<https://blast.ncbi.nlm.nih.gov>) to determine their closest relationship with organism in National Center for Biotechnology Information (NCBI) database. Then, NCBI GenBank accession numbers were assigned to the novel isolates.

Phylogenetic and identity studies

Sequences of selected isolates in NCBI GenBank were aligned with and then trimmed according to sequences of the new Indonesian isolates using ClustalW in MEGA X v.10.2.4 freeware (Kumar et al., 2018). Phylogenetic trees

for *Potyvirus* and *Carlavirus* were constructed by MEGA X using Kimura 2-parameter model (Kimura, 1980) and Maximum Likelihood (ML) statistical method, with 1000 bootstrap replicates to test the statistical significance of each branch. The nucleotide (nt) percentage identities among compared isolates were estimated by Sequence Demarcation Tool (SDT) v1.2 freeware (Muhire et al., 2014).

RESULTS AND DISCUSSION

RT-PCR and virus species determination

The result showed that two samples were infected by *Potyvirus* among three tested potato samples by forming 800 bp bands on agarose gel and none was infected by *Carlavirus*. All three tested shallot samples were infected by *Carlavirus*. The samples formed 715 bp bands on agarose gel but the results were negative for *Potyvirus* (Figure 1). All five isolates were sequenced and the recovered sequences were analyzed using nucleotide BLAST. The result indicated that species of the two *Potyvirus* isolates was PVY and species of the three *Carlavirus* isolates was SLV. The novel Indonesian isolates were deposited in NCBI GenBank and given accession no. OR180052 to OR180053 for PVY and OR180054 to OR180056 for SLV. The PVY caused growth reduction and mosaic on the leaves of infected potato plants, while infected SLV shallots showed mild leaf malformation symptoms (Figure 2). Without presence of OYDV or LYSV, SLV only indicates generating latent or mild symptom on the infected *Allium* spp. (Marais et al., 2019).

Based on the field survey, Kajoran plant disease management practices such as crop rotation, mix cropping, application of plastic

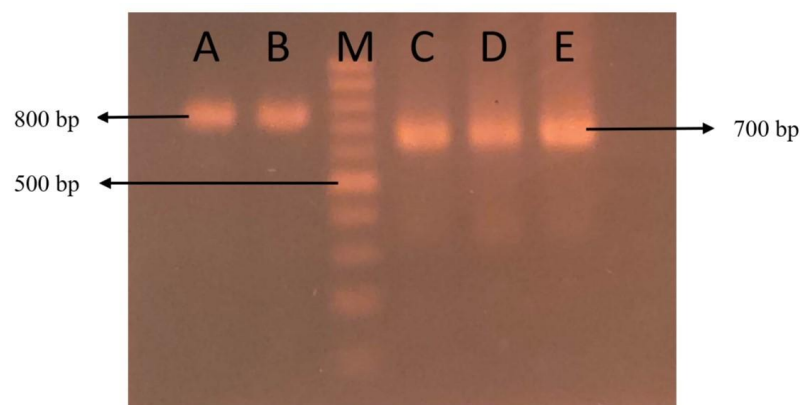


Figure 1. Visualization of RT-PCR amplification results on electrophoresis gel: A) PVY isolates no. OR180052; B) PVY isolate no. OR180053; C) SLV isolate no. OR180054; D) SLV isolate no. OR180055; E) SLV isolate no. OR180056; M) Ladder



Figure 2. Symptoms on infected plants: A) Growth reduction (early stunting) on infected potato plants by PVY isolate no. OR180052; B) Mosaic on the leaf of infected potato plant by PVY isolate no. OR180053; C) Mild leaf malformation on infected shallot by SLV isolate no. OR180054

mulch and removal of weeds were relatively good. Only some potatoes and shallots showed clear viral symptoms. However, most of the symptomatic samples that were taken to laboratory were infected with viruses. The population of aphids, the insect vector of both PVY and SLV (Bhoi et al., 2022; Lou et al., 2023), was observed to be low, likely due to routine insecticide application. The two viruses are also transmitted through vegetative propagation, the main means of potato and shallot transplantation. While SLV is mostly confined to *Allium* spp., PVY is spreadable among a wide host range (Mansouri et al., 2021; Bhoi et al.,

2022). These showed that viral diseases are potentially still hindering crop production in the area.

Phylogenetic study

Two obtained PVY sequences, including 417 bp of 3'-end of CP region, were aligned and tested against 22 isolates in GenBank. Phylogenetic tree showed that the new two PVY isolates shared a basal node with other strain N isolates which was separated from clusters of strains NTN and O. Interestingly, the previous three reported isolates from Sulawesi Island (KT599906 to KT599908) belong to strain NTN,

suggesting quite high genetic variation of Indonesian isolates (Figure 3).

PVY was designated into five major strains: O, C, Z, E and N based on host response and resistance gene interaction. The highly identic isolates to strain N that have recombination in different regions of their genome formed a distinct substrain NTN. Therefore, availability of complete genome sequence is highly recommended in the determination of PVY strains (Singh et al., 2008). Indonesian isolates no. KT599906 to KT599908 inducing mild mosaic on potato were concluded to be part of substrain NTN (Chikh-Ali et al., 2016), meanwhile no. LC384355 inducing stunting on potato plants is concluded to be part of strain O (Fitriyati et al., 2019). LC384355 is potentially found in a mixed infection with other viruses (Fitriyati et al., 2019).

This research presented the first evidence of strain N isolates in Indonesia. The naturally infected potato plant with isolate no. OR180053 showed mosaic on leaf, which was typical symptom of strain N, meanwhile potato plant with isolate no. OR180052 did not show mosaic and showed stunting instead (Figure 2). However, presence of other potato viruses not tested in this study that may influence symptom appearances could not be ruled out. New Indonesian isolates no. OR180052 and OR180053 had the closest phylogenetic relationship with French isolates no. MN216361 and MN216362 from other *Solanum* spp.: tomato (*Solanum lycopersicum*) and weed (*Solanum nigrum*), respectively (Figure 3).

Sequencing of the three SLV isolates recovered 231 bp of 3'-end of CP + 297 bp of

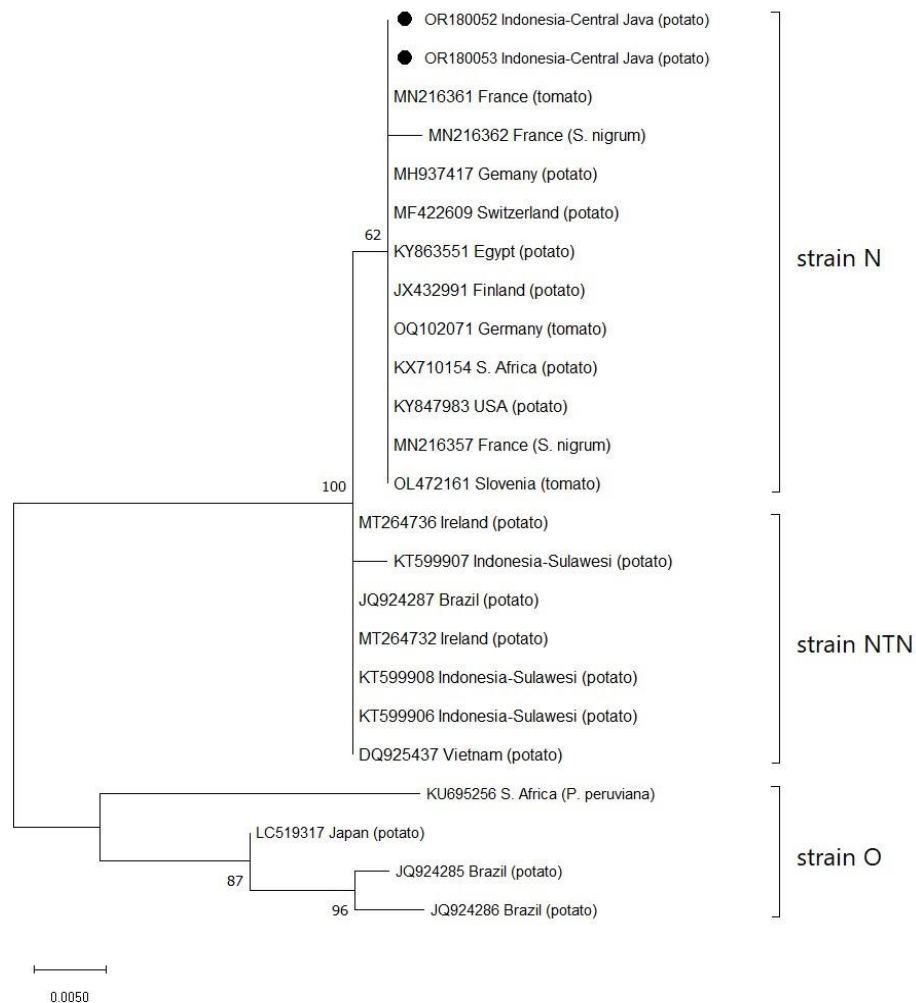


Figure 3. A phylogenetic tree based on 417 bp of 3'-end of CP region of PVY genome was constructed using Kimura 2-parameter model in Maximum Likelihood (ML) statistical method with 1000 bootstraps implemented in MEGA X. Only > 50% of bootstrap values were shown. Black dots indicated the two new Indonesia isolates detected in this study

complete nucleic acid binding protein (NABP) regions (522 bp in total due to slight sequence overlapping). Then these SLV isolates were aligned and compared to 17 isolates in GenBank. The new three shallot isolates were basal sisters with other eight shallot isolates, and formed a distinct phylogroup while isolates from other plant hosts were clustered in several phylogroups (Figure 4). Results of the phylogenetic study indicated that shallot isolates were evolutionary divergent.

Phylogenetic analysis that was performed on partial CP + complete NABP regions demonstrated that the shallot isolates of SLV were genetically different from isolates of garlic and other plant species. The isolates no. OR180054 to OR180056 were positioned closest

to other three Indonesian isolates (KU204906 to KU204908) which were also collected in Java Island (Swari et al., 2015), and one isolate from India (ON624129) (Figure 4). SLV isolates globally show significant evolutionary and genetic divergence, except for those found in Indonesia. However, the situation may be different when isolates from other plant species and outside Java Island are available.

Percentage nucleotide identity

Figure 5 showed that the two PVY isolates from Kajoran shared 100% nt identities between them and also with other ten strain of N isolates, 99.8% with seven isolates of strain NTN and 95.2% with four isolates of strain O at the observed region. SDT analysis calculated 96.4 to 98.5% nt identities among the three SLV

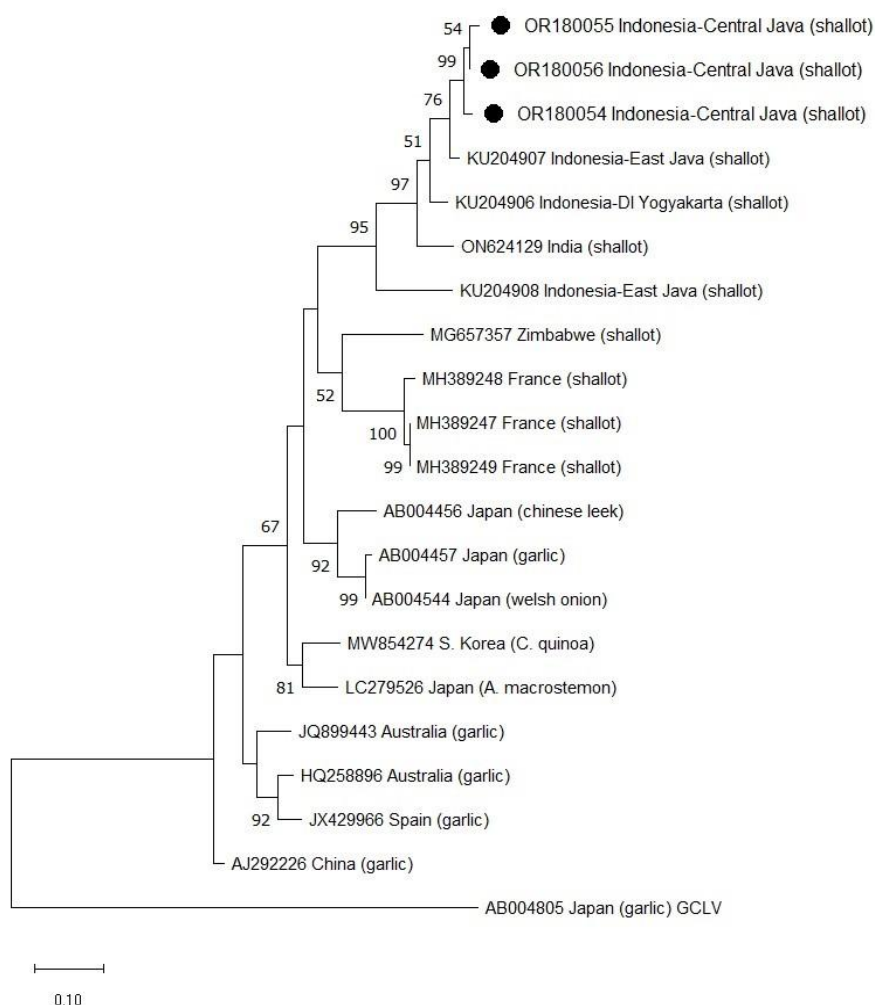


Figure 4. A phylogenetic tree based on 231 bp of 3'-end of CP + 297 bp of complete nucleic acid binding protein (NABP) regions of shallot latent virus genome was constructed using Kimura 2-parameter model in Maximum Likelihood (ML) statistical method with 1000 bootstraps implemented in MEGA X. Only > 50% bootstrap values were shown. Black dots indicated the three new Indonesian isolates detected in this study. A Japanese isolate of garlic common latent virus (GCLV) was used as an out-group

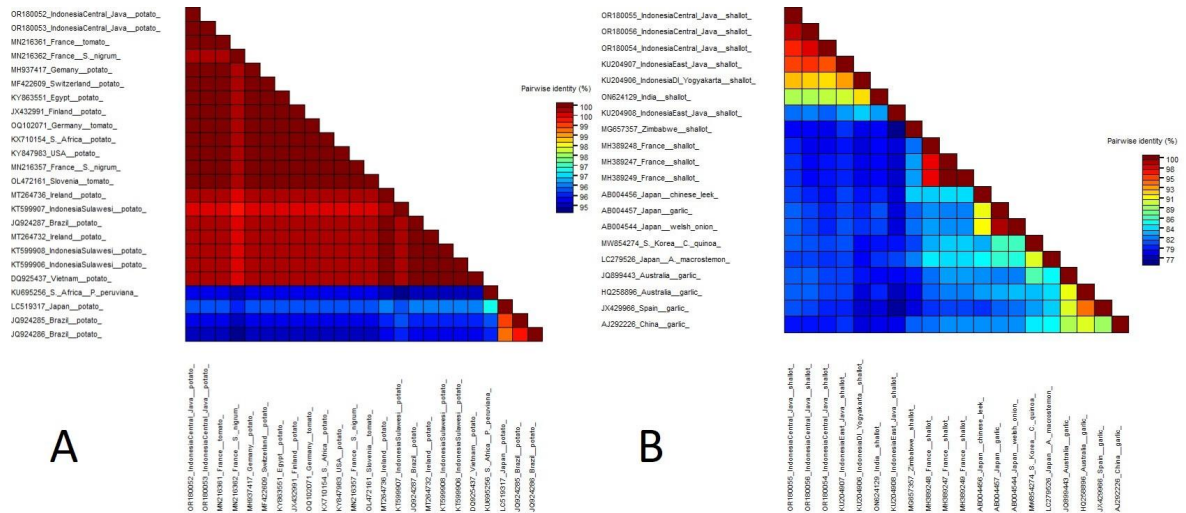


Figure 5. The percentage nucleotide identities of compared isolates were calculated using SDT v1.2. A) PVY; B) SLV

isolates, 78.7 to 95.4% with eight other shallot isolates and 78.9 to 81% with the remaining nine tested isolates. The significant genetic divergence among tested SLV isolates, that was 78.9 to 98.5% nt percentage identities, was caused by frequent variabilities in the sequence of NABP gene.

CONCLUSIONS

This research enriched the study of molecular variation of PVY and SLV isolates in Indonesia. The two obtained PVY isolates belong to strain N group, while the three SLV isolates had the closest relationship with other shallot isolates in the constructed phylogenetic trees. Indonesian isolates need to be fully sequenced in the future to get better understanding of the distribution of PVY strains in the country. The full genome sequences of isolates from *Allium* spp. in Indonesia are also required since it is required to differ in the character of SLV strains or types. Sustainable management for PVY and SLV which involves resistant varieties, certified seeds, bio-insecticide for vector control and crop rotation needs to be developed according to situation in Indonesia.

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