CarakaTani
Journal of Sustainable Agriculture
ISSN 2613-9456 (Print) 2599-2570 (Online)

Caraka Tani: Journal of Sustainable Agriculture, 39(2), 359-369, 2024
URL: https://jurnal.uns.ac.id/carakatani/article/view/86146
DOI: http://dx.doi.org/10.20961/carakatani.v39i2.86146

Molecular Identification of Three Potyviruses Infecting *Allium cepa* var. *aggregatum* and *Allium sativum* in Central Cultivation Areas of Indonesia

Adyatma Irawan Santosa[1*], Muh Amat Nasir[2], Ali Çelik[3], Tahir Farooq[4] and Aprilia Sufi Subiastuti[5]

[1]Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia; [2]Department of Agricultural Socio-Economics, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia; [3]Department of Plant Protection, Faculty of Agriculture, Bolu Abant İzzet Baysal University, Bolu, Türkiye; [4]Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China; [5]Laboratory of Microbiology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

[*]*Corresponding author:* adyatma.i.s@ugm.ac.id

Abstract

One hundred and twenty shallot (*Allium cepa* var. *aggregatum*) and 22 garlic (*Allium sativum*) samples were collected from major growing regions and markets to determine the distribution and molecular diversity of 3 potyviruses: leek yellow stripe virus (LYSV), onion yellow dwarf virus (OYDV), and shallot yellow stripe virus (SYSV) in Indonesia. The results of reverse transcription-polymerase chain reaction (RT-PCR) showed that 83% of shallot and all garlic samples were infected by at least 1 virus species. Coat protein (CP) region of 8 Indonesian LYSV, 19 OYDV, and 10 SYSV isolates were sequenced and given accession nos. OR772038-OR772082 in NCBI GenBank. Five isolates were recombinants according to analysis using the Recombination Detection Program (RDP v5.30). The phylogenetic tree deduced that 6 LYSV Indonesian and 2 China imported isolates belong to S-type. All tested OYDV isolates, including the 19 isolates, were clustered separately according to their respective hosts: onion and garlic. The 10 Indonesian SYSV isolates were clustered together in the same group and thus shown to be closely related. All isolates tested in this study were estimated to be still within their respective species demarcation according to percentage identity analysis. This was the most comprehensive molecular study on LYSV, OYDV, and SYSV that may help to find sustainable management strategies according to conditions in Indonesia and contribute to the global knowledge on the genetic diversity of the 3 viruses.

Keywords: field survey; percentage identity; phylogenetic analysis; recombination event; RT-PCR

Cite this as: Santosa, A. I., Nasir, M. A., Çelik, A., Farooq, T., & Subiastuti, A. S. (2024). Molecular Identification of Three Potyviruses Infecting *Allium cepa* var. *aggregatum* and *Allium sativum* in Central Cultivation Areas of Indonesia. *Caraka Tani: Journal of Sustainable Agriculture*, *39*(2), 359-369. doi: http://dx.doi.org/10.20961/carakatani.v39i2.86146

INTRODUCTION

Each tribe living in the vast Indonesian archipelago may have a distinct culture, which includes culinary taste. However, the majority of menus across the country share a common trait of using shallot (*Allium cepa* var. *aggregatum*) and garlic (*Allium sativum*) as main ingredients thus making both commodities highly valuable. Annual shallot production of around 1,815,445 tons (2020) is enough to cover domestic demand. Central Java, East Java, West Java, and Yogyakarta Provinces, all in Java Island, contribute 72% of the national output, some of which are then distributed to provinces in other islands. Sumatera Island also has a considerable

[*] *Received for publication April 22, 2024*
Accepted after corrections August 5, 2024

proportion of shallot production. On the other hand, cultivation centers in the country are estimated to produce only around 81,805 tons year^{-1} (2020) due to the difficulty of growing garlic in tropical settings. As a consequence, no less than 500,000 tons of garlic are imported to Indonesian markets each year, mostly from China. *Allium* spp. are also grown commercially around the world.

Due to the nature of shallot and garlic vegetative propagations, viruses from the genera *Potyvirus*, *Carlavirus*, *Allexivirus*, and *Tospovirus* are commonly found infecting both horticultural produces worldwide (Abraham et al., 2019; Santosa and Ertunc, 2020; Cremer et al., 2021). Mixed infection of potyviruses and carlaviruses, as a 'garlic viral complex', could produce more severe symptoms than a single infection (Fajardo et al., 2001). Accumulation of viruses during 5 years of consecutive plantings has also been observed to reduce garlic bulb weight by up to 49% (Conci et al., 2003).

Members of potyvirus, leek yellow stripe virus (LYSV) and onion yellow dwarf virus (OYDV), have a worldwide presence (Fajardo et al., 2001; Ward et al., 2009; Parrano et al., 2012) while shallot yellow stripe virus (SYSV) seems to be mostly confined to East Asia (China, Japan, and South Korea) and South East Asia (Indonesia, Vietnam, and Laos) (Van Der Vlugt et al., 1999; Chittarath et al., 2017; Yang et al., 2019) although Mansouri et al. (2021) recently reported SYSV in the Czech Republic. The 3 virus species have been detected in shallot and garlic as well as other important *Allium* spp., including onion (*A. cepa*), Welsh onion (*A. fistulosum*), and leek (*A. ampeloprasum*).

Garlic infected by OYDV shows mild until severe deformation on its leaf and suffers high yield losses of up to 60% (Lot et al., 1998; Conci et al., 2003). Similarly, LYSV causes the typical yellow stripes symptom on leaves of *Allium* spp., reported to reduce garlic production to 54% (Lot et al., 1998; Lunello et al., 2002). Besides spreading vegetatively, the 3 potyviruses are also disseminated by several aphid species (Lunello et al., 2002; Jayasinghe et al., 2021), further complicating the disease management.

The flexuous elongated thread-like particles of potyviruses are measured around 10 to 20 nm in diameter and 700 to 800 nm in length (Mahmoud et al., 2007). The full length of genomes of LYSV, OYDV, and SYSV, similar to other species of potyvirus, is around 10 kbp. The genome is single-stranded RNA (ssRNA) with a single long open reading frame (ORF) encoding 1 large polyprotein that can be further separated into 10 small proteins, including coat protein (CP) (Verma et al., 2015; Gupta et al., 2017). Phylogenetic analyses on the 3 potyviruses infecting *Allium* spp. were usually based on their CP encoding region which are determined to be highly conserved (Cremer et al., 2021; Tuzlali et al., 2021). Particularly, observation on the CP sequences of LYSV showed that global population of the virus can be genetically separated into S, L, and N types (Santosa et al., 2023).

LYSV, OYDV, SYSV and other viruses were found in shallot and garlic in Indonesia during few small-scale surveys (Harti et al., 2020; Nurenik et al., 2021; Hidayat et al., 2023; Santosa et al., 2024a). As of January 2024, nucleotide sequences of only 1 LYSV, 3 SYSV, and 13 OYDV isolates from Indonesia have been listed in NCBI GenBank thus distribution and genetic variation of these viruses in the country are still largely unknown. The nucleotide sequences of CP region obtained here were analyzed in the 1st comprehensive molecular study to expand our understanding on the diversity of shallot and garlic isolates of LYSV, OYDV, and SYSV from major cultivation centers in Indonesia and thus may also contribute valuable data on the phylogenies of the 3 important *Allium* viruses.

MATERIALS AND METHOD

Field surveys

Shallot samples were collected from production centers in West Java, Central Java, East Java, and Yogyakarta Provinces in Java Island as well as North Sumatera, South Sumatera, and Bengkulu Provinces in Sumatera Island. Garlic samples were taken only from locations in Central Java Province, including Semarang, Magelang, and Karanganyar Regencies, due to limited cultivation in other regions of Indonesia. Due to many latent infections and similarities between symptoms caused by viruses and other factors in *Allium* spp., samples were taken randomly to represent different planting spots in each field. Symptomatic plants showing chlorosis, leaf malformation, wilting, and stunting were prioritized but non-symptomatic samples were also collected. The distance of sampled fields was at least 100 m from each other. Number of the collected samples was in line with the size of the fields, and the total collected samples in each

province corresponded to the approximate total production of the province. Garlic cloves imported from China were randomly taken from markets to complement the genetic diversity analysis. Samples were brought to the Phytopathology Laboratory, Universitas Gadjah Mada, and stored at 4 °C until further testing.

Total RNA extraction and RT-PCR

Total RNAs were extracted from bulbs of shallot and garlic samples using Plant Total RNA Mini Kit (Geneaid Biotech Ltd., Taiwan) following the kit's standard protocols. cDNAs were synthesized from the extracted RNA using ReverTra Ace kit (Toyobo, Japan) in a reverse transcription reaction volume of 10 µl: 2 µl RNA, 1 µl dNTP, 0.5 µl (10 pmol μl^{-1}) Oligo(dT) primer, 0.5 µl RNAse inhibitor, 0.5 µl ReverTraAce®, 2 µl 5× RT Buffer, and 3.5 µl nuclease-free water. The reverse transcription steps were performed at 42 °C for 20 minutes, followed by 99 °C for 5 minutes.

The subsequent PCRs were each performed in a reaction volume of 40 µl: 20 µl of MyTaq HS Red Mix (Bioline, Germany), 2 µl (10 pmol μl^{-1}) each of the reverse and the forward primers for specific amplification of CP regions of LYSV, SYSV, or OYDV (Table 1), 4 µl of cDNA, and 12 µl of PCR-grade water. The thermal cycler program was 95 °C for 3 minutes as a pre-denaturation, 35 cycles of denaturation at 95 °C for 1 minute, annealing at (Table 1), and elongation at 72 °C for (Table 1), followed by final elongation at 72 °C for 10 minutes.

The appearance of specific target bands on 1% agarose gel stained with Florosafe DNA Staining (1st BASE, Malaysia) after electrophoresis for 50 minutes at 100 V was observed using a UV transilluminator (Optima Inc., Japan). The successfully amplified PCR products were sent to a biotechnological firm (1st BASE, Malaysia) for dual-directional Sanger Sequencing using species-specific primers (Table 1). The obtained nucleotide sequences were then run using nucleotide BLAST online software (https://blast.ncbi.nlm.nih.gov) to determine their highest similarities with organisms registered in the NCBI database. After that, NCBI GenBank accession numbers were obtained for the sequences of isolates.

Recombination, phylogenetic, and percentage identity analyses

Sequences of selected isolates listed in NCBI GenBank were aligned with and then trimmed according to the recovered nucleotide sequences of isolates using ClustalW option in MEGA11 v11.0.13 software (Tamura et al., 2021). Each alignment was separately scanned by the recombination detection program (RDP v5.30) to detect possible recombinants among tested isolates (Martin et al., 2021). Only significant recombination events with support by at least 5 of the algorithms implemented in RDP v5.30 (Bonferroni-corrected *p*-value of < 0.05) were reported in this study.

The phylogenetic tree for each species of potyvirus was constructed in MEGA11 using maximum likelihood (ML) statistical method, Tamura-Nei-parameter model (confirmed as the best-supporting DNA model for all 3 alignments by MEGA11) with 1,000 bootstrap replicates, and uniform rates among sites (Tamura and Nei, 1993). The percentage similarities among

Table 1. List of primers used in the PCR detection of LYSV, OYDV, and SYSV, their target gene, annealing temperatures and times, and product size

Virus	Target gene	Sequence (5′–3′)	Annealing temp. and time	Expected product size (bp)	References
LYSV	Partial NIb + complete CP	F-TCACTGCATATGCGCACCAT R-GCACCATACAGTGAATTGAG	50 ºC for 60 seconds	1,020	Fajardo et al. (2001)
OYDV	Partial CP	F-YGTYGAYRCTGGMACHACYG R-RTTACCATCMARGCCAAACA	57 ºC for 60 seconds	615	Manglli et al. (2014)
SYSV	Partial CP	F-GCAGGATCCAACACCRAGTTATGTGTC R-TTCGGATCCATRTGAGCTTCCTTCGC	52 ºC for 30 seconds	700	Van Der Vlugt et al. (1999)

Note: R = A or G, Y = C or T, and H = A or T or C

sequences in each alignment at nucleotide (nt) and amino acid (aa) levels were estimated by sequence demarcation tool (SDT v1.2) (Muhire et al., 2014).

RESULTS AND DISCUSSION

A total 120 shallot samples were collected from production centers in Java Island: Bandung, West Java Province (15 samples from 3 fields); Brebes, Central Java Province (33, 10 fields); Magelang, Central Java Province (17, 5 fields); Nganjuk, East Java Province (18, 5 fields); and Kulon Progo, Yogyakarta Province (20, 7 fields) as well as in Sumatera Island: Berastagi, North Sumatera Province (5, 2 fields); Ogan Ilir, South Sumatera Province (7, 2 fields); and Kepahiang, Bengkulu Province (5, 2 fields) (Figure 1).

Garlic samples were taken from cultivation areas in Central Java Province, including Semarang (2 samples from 1 field), Magelang (2, 1 field), and Karanganyar Regencies (16, 4 fields). Two garlic cloves imported from China were each randomly taken from markets in Sleman, Yogyakarta Province, and Semarang, Central Java Province, to bring the total number of garlic samples to 22 (Figure 1).

RT-PCR showed that 27 of the 120 shallot samples were positive for OYDV, 14 for SYSV, and 59 for OYDV + SYSV mixed infection. Besides that, 20 samples were tested negative for all 3 viruses, and none were confirmed to be infected by LYSV. The molecular test also detected LYSV and OYDV as single infections in 2 and 5 local garlic samples, respectively. Mixed infections of LYSV and OYDV were found in 13 of the tested local garlic samples. Both garlic samples imported from China were tested positive for LYSV only. Therefore, all of the garlic samples were confirmed to be infected by at least 1 virus (Table 2). Most infected local garlic samples showed stunted growth while a few samples exhibited no clear symptoms. Similarly, both LYSV-infected samples imported from China looked healthy and were marketed at high prices. Eight LYSV, 19 OYDV, and 10 SYSV (37 in total) isolates were selected based on different hosts and sampling locations for partial genome sequencing. The obtained sequences were then registered in NCBI GenBank to secure accession nos. OR772038-OR772074.

Shallot and garlic in Indonesia are mostly grown vegetatively thus, as expected, heavily infected by viruses. Transmission of by aphid vectors probably also helps to spread the 3 potyviruses. However, there were not any aphids in fields during this survey, likely due to rather intensive application of insecticides. Only 20 out of 120 shallot samples (17%) were free from the 3 potyviruses, and none of the garlic samples were tested negative. Samples from each of 7 provinces were found to be infected by at least 1 virus species. Most of the infected shallots exhibited stunting or leaf yellowing (chlorosis). Some symptomless samples or those exhibiting symptoms that may can be attributed to other factors, such as wilting and rotting, were also tested positive for virus infection. On the contrary, some samples with typical viral symptoms such as chlorosis, stunting, or leaf deformation were negative for the tested viruses. These results indicated an expansive distribution of viruses in all major production areas of Indonesia. Diversity analysis to learn the structure of virus population in Indonesia in comparison with those of other countries needs to be established in the future (Akbaş et al., 2023).

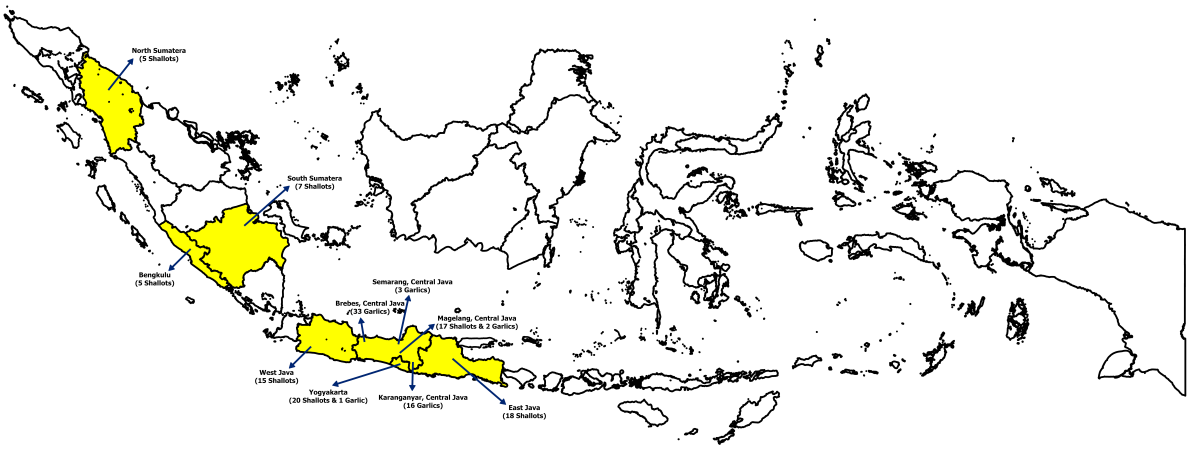

Figure 1. Sampling locations in main production centers of shallot and garlic in Indonesia

Table 2. Result of molecular identification of LYSV, OYDV, and SYSV in shallot and garlic samples collected in central cultivation areas of Indonesia

Shallot sampling areas	LYSV	OYDV	SYSV	LYSV + OYDV	LYSV + SYSV	OYDV + SYSV	OYDV + LYSV + SYSV	Negative	Total
N. Sumatera	0	3	1	0	0	1	0	0	5
S. Sumatera	0	1	1	0	0	5	0	0	7
Bengkulu	0	1	2	0	0	2	0	0	5
W. Java Bandung	0	3	1	0	0	8	0	3	15
C. Java Magelang	0	2	2	0	0	10	0	3	17
C. Java Brebes	0	12	1	0	0	15	0	5	33
E. Java Nganjuk	0	3	2	0	0	7	0	6	18
Yogyakarta Kulon Progo	0	2	4	0	0	11	0	3	20
Total	0	27	14	0	0	59	0	20	120
Garlic sampling areas	LYSV	OYDV	SYSV	LYSV + OYDV	LYSV + SYSV	OYDV + SYSV	OYDV + LYSV + SYSV	Negative	Total
C. Java Semarang	1	1	0	0	0	0	0	0	2
C. Java Magelang	0	1	0	1	0	0	0	0	2
C. Java Karanganyar	1	3	0	12	0	0	0	0	16
China: import	2	0	0	0	0	0	0	0	2
Total	4	5	0	13	0	0	0	0	22

LYSV was not detected in any of 120 shallot samples while 17 of 22 garlic samples tested in this study (77%) was found to be infected by the virus. This result was actually in line with previous reports that also only identified OYDV and SYSV in shallots grown in Sumatera, Java, Nusa Tenggara, and Sulawesi Islands (Harti et al., 2020; Nurenik et al., 2021). The primer pair specific for LYSV used in the current molecular assay had been previously applied successfully in the detection of S-type isolates from onion, garlic, and leek (Tuzlali et al., 2021) and L-type isolates from leek and garlic (Santosa et al., 2023). Tuzlali et al. (2021) reported that LYSV was identified only in 3 out of 197 onion samples from 3 Turkish provinces. Therefore, it can be suggested that LYSV distribution in onion and shallot is limited.

RDP5 analysis detected significant recombination events on the CP sequences of several isolates. Recombinant LYSV OR772045 received donors from Chinese and Brazilian isolates while OR772040 got from Chinese and other Indonesian isolates. LYSV OR772038 was found on garlic imported from China and deduced to receive donor sequences from Chinese and Japanese isolates. Only 1 OYDV (OR772056) and 1 SYSV (OR772050) were found to have recombination events, with parent isolates from Spain and Indonesia (Table 3).

The recombination in Indonesian isolates No. OR772040, OR772050, and OR772056 likely happened locally since their parental donors were other Indonesian isolates. Similarly, recombinant isolate No. OR772038, which was obtained from garlic imported from China, has Chinese and Japanese parents. OR772038 and another from garlic imported from China, OR772039, together with 3 Chinese isolates, occupied a subgroup distinct to the subgroup where Indonesian isolates belong in the LYSV phylogenetic tree, further underlining genetic divergence between local and imported isolates. Although the tested 2 imported garlics were only positive for LYSV, it is very likely other imported garlic carries various

Table 3. Putative recombination events detected by RDP v5.30 analysis on the CP region of isolates obtained in this study

No.	Recombinant	Parents: major/minor	Breakpoints[1] (start/end)	RDP implemented method[2] (*p*-value)
LYSV				
1.	OR772045 (Central Java, Indonesia)	MT358343 (China)/ ON565071 (Brazil)	694/806	R ($2.571x10^{-07}$) G ($7.269x10^{-04}$) B ($2.175x10^{-06}$) M ($2.994x10^{-04}$) C ($1.867x10^{-02}$) S ($1.853x10^{-27}$) 3S ($6.990x10^{-06}$)
2.	OR772040 (Central Java, Indonesia)	MT358343 (China)/ MW854277 (Indonesia)	647/749	G ($3.053x10^{-04}$) B ($6.726x10^{-04}$) M ($2.451x10^{-03}$) C ($4.909x10^{-02}$) S ($5.836x10^{-32}$) 3S ($2.432x10^{-04}$)
3.	OR772038 (China import, Indonesia)	MN059477 (China)/ AB194636 (Japan)	556/766	R ($1.422x10^{-17}$) G ($4.023x10^{-15}$) B ($5.080x10^{-16}$) M ($2.394x10^{-15}$) C ($1.753x10^{-12}$) S ($2.222x10^{-23}$) 3S ($6.365x10^{-25}$)
OYDV				
1.	OR772056 (Central Java, Indonesia)	JX429964 (Spain)/ OR772060 (West Java, Indonesia)	33/533	R ($2.742x10^{-05}$) G ($5.059x10^{-04}$) B ($2.311x10^{-04}$) C ($3.291x10^{-02}$) 3S ($2.719x10^{-04}$)
SYSV				
1.	OR772050 (Central Java, Indonesia)	OR772053 (Central Java, Indonesia)/ OR772055 (Central Java, Indonesia)	223/523	R ($6.053x10^{-04}$) G ($1.563x10^{-02}$) B ($2.659x10^{-04}$) M ($5.871x10^{-08}$) C ($5.182x10^{-08}$) S ($2.715x10^{-11}$) 3S ($2.623x10^{-10}$)

Note: [1, 2] = Position in alignment; R = RDP; G = GENECOV; B = BootScan; M = MaxChi; C = Chimaera; S = Siscan; 3S = 3Seq

viruses. The vast majority of garlic imported to Indonesia from China is intended for consumption. The very limited cultivation in Indonesia usually uses local garlic varieties which, including those sampled in this study, are morphologically smaller in size than imported garlic. Despite those facts, there remains a risk of introducing new virus strains as the garlic is imported fresh and thus still viable as propagation material. This report provides the 1st insights into viruses carried by garlic imported from China to Indonesia.

All 6 Indonesian and 2 Chinese imported isolates belong to 1 of the major LYSV phylogenies, S-type. All Indonesian isolates are in a subgroup together with isolates from Chile, China, Turkey, Mexico, and Iran. In a separate subgroup, the 2 Chinese imported isolates have the closest relationship with 3 other isolates also from China, including Hohhot1, Yuhang GHY,

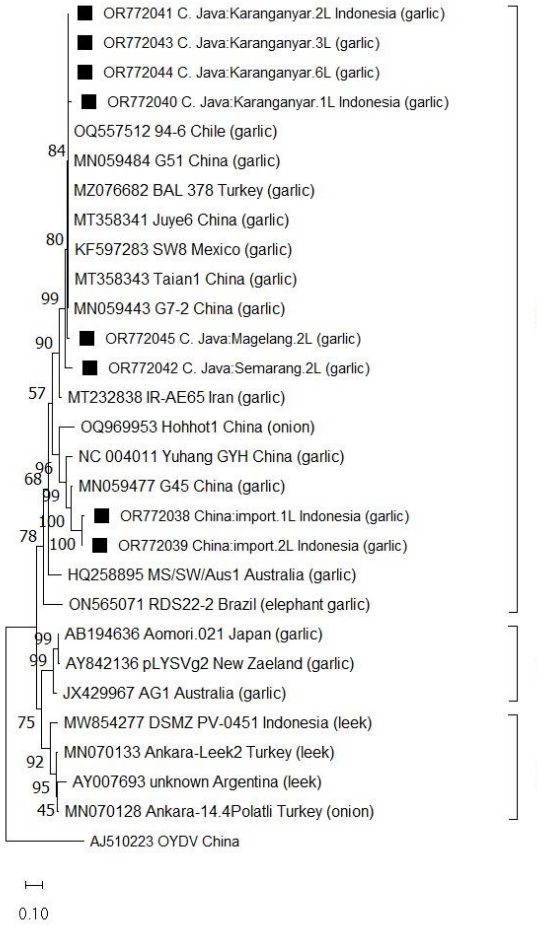

Figure 2. A phylogenetic tree based on the complete CP region (864-867 bp) of the LYSV genome was constructed using Tamura-Nei-parameter model in maximum likelihood statistical method with 1,000 bootstraps suited in MEGA11 software

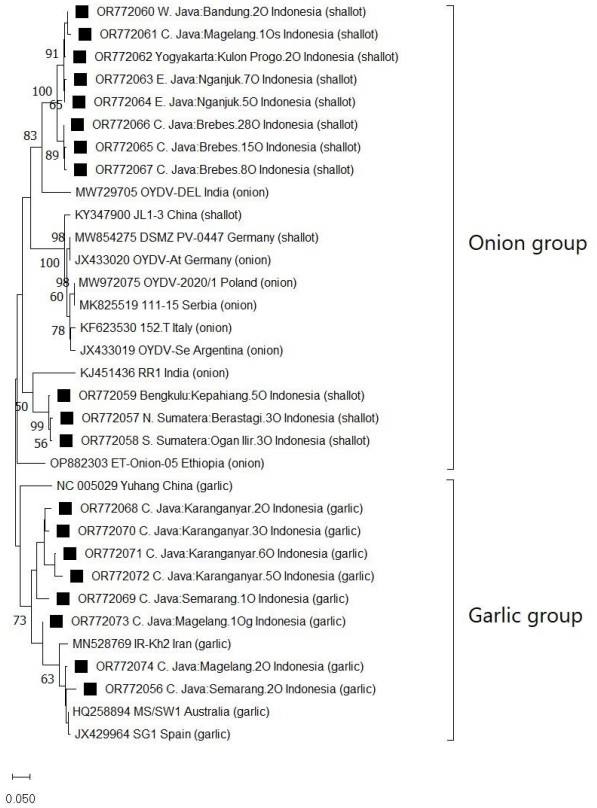

Figure 3. A phylogenetic tree based on partial CP region (687 of 771 bp) of the OYDV genome was constructed using Tamura-Nei-parameter model in maximum likelihood statistical method with 1,000 bootstraps suited in MEGA11 software

Note: Only bootstrap values greater than 50% were shown. Black squares pointed to the isolates were characterized in this study

and G45 (Figure 2). These results indicated that the phylogroup of LYSV was related more to host species than the geographic origins of isolates, in agreement with previous analysis (Santosa et al., 2023).

Analysis of the obtained partial sequences of CP of OYDV resulted in a phylogenetic tree that separated the tested isolates into 2 main groups based on their hosts, which are onion + shallot, and garlic. Eight isolates from shallot samples collected on Java Island were positioned in a subgroup with an Indian isolate (OYDV-DEL) while 3 shallot isolates from Sumatera Island were included in a separate subgroup with another Indian isolate (RRI) (Figure 3).

The 8 garlic isolates were taken from several neighboring regencies in Central Java Province and shown to be genetically closely related as they were all clustered together in a subgroup. Isolates from Iran (IR-Kh2), Australia (MS/SW1), and Spain (SG1) were also in the same subgroup while an isolate from China (Yuhang) formed a distinct monophyletic subgroup within the garlic group (Figure 3). Genetic adaptation of OYDV to hosts, leading to isolates separation into onion and garlic phylogroups, was also reported in other studies (Cremer et al., 2021; Santosa et al., 2024b).

The tested SYSV isolates formed 2 separate groups in the phylogenetic tree constructed on a partial sequence of CP. All 10 isolates from both Java and Sumatera Islands were close to each other and clustered in Group 1 with isolates from Japan and Laos (Q6489), and another from Indonesia (DSMZ PV-0452) (Figure 4).

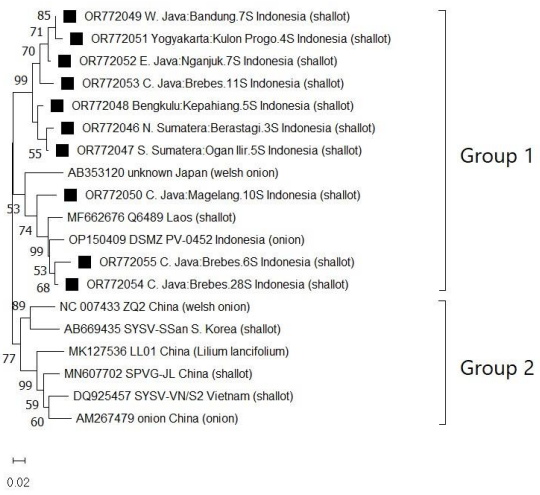

Figure 4. A phylogenetic tree based on partial CP region (638 of 774 bp) of the SYSV genome was constructed using Tamura-Nei-parameter model in maximum likelihood statistical method with 1,000 bootstraps suited in MEGA11 software

Note: Only bootstrap values greater than 50% were shown. Black squares pointed to the isolates were characterized in this study

The compared full CP of LYSV isolates shared 74.4 to 99.9% and 74.1 to 100% identities among them at nt and aa levels, respectively. The SDT analysis also found that the OYDV isolates had 81.8 to 99.2% nt and 87.8 to 99.5% aa identities, and SYSV isolates had 86.4 to 98.6% nt and 92.1 to 99.1% aa identities among them based on partial CP comparisons. Among other criteria, isolates with > 76 to 77% nt and > 80% aa identities at CP region should be considered as the same species within the genus potyvirus (Adams et al., 2005).

SYSV is the least studied among 3 potyviruses infecting *Allium* spp., as it was mostly reported in shallot and onion in East and South East Asia (Van Der Vlugt et al., 1999; Chittarath et al., 2017; Yang et al., 2019; Harti et al., 2020). However, the virus could spill into new territories due to rapid global trade. SYSV has recently been detected in the Czech Republic (Mansouri et al., 2021). Looking back, 2 Indonesian shallot isolates (AB000842 and AB000844) were among the 1st observed to distinguish SYSV as a species distinct from its closest relative, OYDV (Tsuneyoshi et al., 1998; Van Der Vlugt et al., 1999). This current study confirmed that the virus was still widespread in the country as it was detected in 73 out of 120 shallot samples (61%) (Table 2). Another paper also published a high incidence of up to 93% of SYSV in shallot fields in Indonesia (Harti et al., 2020). Analysis of the partial sequence of CP region divided worldwide isolates into 2 main groups. Indonesian isolates from Sumatera and Java Islands showed low divergence among them and were all clustered in Group 1 (Figure 4). Therefore, additional important molecular variability data on SYSV were reported here.

CONCLUSIONS

This study presented the most comprehensive survey on 3 potyviruses infecting shallot and garlic cultivated in major production areas in Sumatera and Java Islands of Indonesia. The obtained molecular data expanded the knowledge of the diversity of potyviruses that have caused high losses among shallot and garlic around the world. However, future surveys should include carlaviruses and allexiviruses to complement our understanding of the genetic variation among *Allium* viruses in Indonesia. Identity analysis suggested that all isolates tested in this study are still within their respective species demarcation. A high incidence of all 3 viruses should alert stakeholders to quickly find viable management to reduce yield losses in Indonesia, which may include the production of 'true shallot seed' and virus-free seedlings through tissue culture. The possible introduction of new virus strains to the country via imported garlic also needs to be addressed.

ACKNOWLEDGEMENT

This research was supported by the Doctoral Competency Improvement Program of Universitas Gadjah Mada, under grant number 7743/UN1.P.II/Dit-Lit/PT.01.03/2023. The authors declare no financial conflicts of interest or personal relationships that could have influenced the work presented in this paper. The authors would like to thank Alifia Nur Ayusma (Universitas Gadjah Mada), Reko Saputra Jaya (Universitas Gadjah Mada), Efriandi (Department of Agriculture of South Sumatera Province), and Benny Ajie Wijaya (A private agricultural company in North Sumatera Province) for their assistance in sample collection.

REFERENCES

Abraham, A. D., Kidanemariam, D. B., & Holton, T. A. (2019). Molecular identification, incidence and phylogenetic analysis of seven

viruses infecting garlic in Ethiopia. *European Journal of Plant Pathology*, *155*(1), 181–191. https://doi.org/10.1007/s10658-019-01760-9

Adams, M. J., Antoniw, J. F., & Fauquet, C. M. (2005). Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Archives of Virology*, *150*(3), 459–479. https://doi.org/10.1007/s00705-004-0440-6

Akbaş, B., Morca, A. F., Coşkan, S., Santosa, A. I., Çulal-Kılıç, H., & Çelik, A. (2023). First complete sequences and genetic variation of plum pox virus T strain in *Prunus dulcis* and *Prunus cerasus*. *3 Biotech*, *13*(10), 332. https://doi.org/10.1007/s13205-023-03746-1

Chittarath, K., Rungsawang, W., Pongsapich, P., Kong, G. A., Thomas, J. E., & Geering, A. D. W. (2017). First records of the potyviruses *Chilli ringspot virus* and *Shallot yellow stripe virus* from Laos. *Australasian Plant Disease Notes*, *12*, 53. https://doi.org/10.1007/s13314-017-0278-x

Conci, V. C., Canavelli, A., & Lunello, P. (2003). Yield losses associated with virus-infected garlic plants during five successive years. *Plant Disease*, *87*(12), 1411–1415. https://doi.org/10.1094/pdis.2003.87.12.1411

Cremer, J., Campbell, P., Steele, V., Persley, D., Thomas, J., Harper, S., & Gambley, C. (2021). Detection and distribution of viruses infecting garlic crops in Australia. *Plants*, *10*(5), 1013. https://doi.org/10.3390/plants10051013

Fajardo, T. V. M., Nishijima, M., Buso, J. A., Torres, A. C., Ávila, A. C., & Resende, R. O. (2001). Garlic viral complex: Identification of potyviruses and carlavirus in Central Brazil. *Fitopatologia Brasileira*, *26*(3), 619–626. https://doi.org/10.1590/S0100-41582001000300007

Gupta, N., Islam, S., Sharma, S. K., & Baranwal, V. K. (2017). Complete genome sequence of an isolate of leek yellow stripe virus from garlic in India. *Journal of Plant Pathology*, *99*(3), 793–797. Retrieved from https://www.jstor.org/stable/44687156

Harti, H., Hidayat, S. H., Sobir, & Wiyono, S. (2020). Detection of major viruses infecting shallot and molecular characterization of onion yellow dwarf virus from several locations in Indonesia. *Biodiversitas Journal of Biological Diversity*, *21*(4), 1697–1701. https://doi.org/10.13057/biodiv/d210451

Hidayat, S. H., Meliyana, Yulianingsih, R., Dinarti, D., & Nurulita, S. (2023). Incidence of main viruses infecting local garlic in Java, Indonesia. *Jurnal Hama dan Penyakit Tumbuhan Tropika*, *23*(2), 7–15. https://doi.org/10.23960/jhptt.2237-15

Jayasinghe, W. H., Kim, H., Sasaki, J., & Masuta, C. (2021). Aphid transmissibility of onion yellow dwarf virus isolates with an N-terminal truncated HC-Pro is aided by leek yellow stripe virus. *Journal of General Plant Pathology*, *87*(3), 178–183. https://doi.org/10.1007/s10327-021-00986-y

Lot, H., Chovelon, V., Souche, S., & Delecolle, B. (1998). Effects of onion yellow dwarf and leek yellow stripe viruses on symptomatology and yield loss of three French garlic cultivars. *Plant Disease*, *82*(12), 1381–1385. https://doi.org/10.1094/PDIS.1998.82.12.1381

Lunello, P., Ducasse, D. A., Helguera, M., Nome, S. F., & Conci, V. C. (2002). An Argentinean isolate of leek yellow stripe virus from leek can be transmitted to garlic. *Journal of Plant Pathology*, *84*(1), 11–17. Retrieved from https://www.jstor.org/stable/41998075

Mahmoud, S. Y. M., Abo-El Maaty, S. A., El-Borollosy, A. M., & Abdel-Ghaffar, M. H. (2007). Identification of onion yellow dwarf potyvirus as one of the major viruses infecting garlic in Egypt. *International Journal of Virology*, *4*(1), 1–13. https://doi.org/10.3923/ijv.2008.1.13

Manglli, A., Mohammed, H., EL Hussein, A., Agosteo, G., Albanese, G., & Tomassoli, L. (2014). Molecular analysis of the 3' terminal region of onion yellow dwarf virus from onion in southern Italy. *Phytopathologia Mediterranea*, *53*(3), 438–450. Retrieved from https://www.jstor.org/stable/43871796

Mansouri, F., Krahulec, F., Duchoslav, M., & Ryšánek, P. (2021). Newly identified host range of viruses infecting species of the genus *Allium* and their distribution in six habitats in the Czech Republic. *Plant Pathology*, *70*(6), 1496–1507. https://doi.org/10.1111/ppa.13391

Martin, D. P., Varsani, A., Roumagnac, P., Botha, G., Maslamoney, S., Schwab, T., Kelz, Z.,

Kumar, V., & Murrell, B. (2021). RDP5: A computer program for analyzing recombination in, and removing signals of recombination from, nucleotide sequence datasets. *Virus Evolution*, *7*(1), veaa087. https://doi.org/10.1093/ve/veaa087

Muhire, B. M., Varsani, A., & Martin, D. P. (2014). SDT: A virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS ONE*, *9*(9), e108277. https://doi.org/10.1371/journal.pone.0108277

Nurenik, N., Hartono, S., Sulandari, S., Somowiyarjo, S., & Kandito, A. (2021). Double infection of onion yellow dwarf virus and shallot latent virus in garlic from several regions in Indonesia. *Jurnal Perlindungan Tanaman Indonesia*, *25*(1), 40–47. https://doi.org/10.22146/jpti.62818

Parrano, L., Afunian, M., Pagliaccia, D., Douhan, G., & Vidalakis, G. (2012). Characterization of viruses associated with garlic plants propagated from different reproductive tissues from Italy and other geographic regions. *Phytopathologia Mediterranea*, *51*(3), 549–565. Retrieved from https://www.jstor.org/stable/43872342

Santosa, A. I., & Ertunc, F. (2020). Identification, molecular detection and phylogenetic analysis of four viruses infecting *Allium cepa* in Ankara Province, Turkey. *Journal of Plant Diseases and Protection*, *127*(4), 561–569. https://doi.org/10.1007/s41348-020-00347-5

Santosa, A. I., Irbati, A. H., Dharma, K. S., Winona, B., Jaya, R. S., Andriyani, A. L., & A'yun, C. Q. (2024a). Potato virus Y and shallot latent virus of Kajoran Horticultural Production Center, Magelang Regency, Indonesia: Molecular characterization case study. *Caraka Tani: Journal of Sustainable Agriculture*, *39*(1), 1–9. https://doi.org/10.20961/carakatani.v39i1.76737

Santosa, A. I., Ningrum, D. R., Yuliandini, S. N. S., Ayusma, A. N., Nasir, M. A., & Subiastuti, A. S. (2024b). Molecular identification of onion yellow dwarf virus in *Allium fistulosum* and *Allium schoenoprasum* in Indonesia. *Pakistan Journal of Phytopathology*, *36*(1), 87–93. https://doi.org/10.33866/phytopathol.036.01.1054

Santosa, A. I., Randa-Zelyüt, F., Karanfil, A., Korkmaz, S., Hartono, S., & Ertunç, F. (2023). Phylogenetic and diversity analyses revealed that leek yellow stripe virus population consists of three types: S, L, and N. *Virus Genes*, *59*(1), 121–131. https://doi.org/10.1007/s11262-022-01956-y

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, *10*(3), 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120

Tsuneyoshi, T., Matsumi, T., Natsuaki, K. T., & Sumi, S. (1998). Nucleotide sequence analysis of virus isolates indicates the presence of three potyvirus species in *Allium* plants. *Archives of Virology*, *143*(1), 97–113. https://doi.org/10.1007/s007050050271

Tuzlali, H. T., Karanfil, A., & Korkmaz, S. (2021). Occurrence of leek yellow stripe virus and onion yellow dwarf virus from edible *Allium* plants in the south Marmara region of Turkey. *3 Biotech*, *11*(12), 516. https://doi.org/10.1007/s13205-021-03067-1

Van Der Vlugt, R. A. A., Steffens, P., Cuperus, C., Barg, E., Lesemann, D.-E., Bos, L., & Vetten, H. J. (1999). Further evidence that shallot yellow stripe virus (SYSV) is a distinct potyvirus and reidentification of Welsh onion yellow stripe virus as a SYSV strain. *Phytopathology*, *89*, 148–155. https://doi.org/10.1094/phyto.1999.89.2.148

Verma, R. K., Mishra, R., Petrov, N. M., Stoyanova, M., Stoev, A., Bakardjieva, N., & Gaur, R. K. (2015). Molecular characterization and recombination analysis of an Indian isolate of onion yellow dwarf virus. *European Journal of Plant Pathology*, *143*(3), 437–445. https://doi.org/10.1007/s10658-015-0695-7

Ward, L. I., Perez-Egusquiza, Z., Fletcher, J. D., & Clover, G. R. G. (2009). A survey of viral diseases of *Allium* crops in New Zealand. *Australasian Plant Pathology*, *38*(5), 533–539. https://doi.org/10.1071/AP09039

Yang, P. P., He, G. R., Cao, Y. W., Xu, L. F., & Ming, J. (2019). First report of *shallot yellow stripe virus* in *Lilium lancifolium* in China. *Plant Disease*, *103*(8), 2145. https://doi.org/10.1094/PDIS-11-18-1994-PDN