

RESEARCH PAPER

Incidence of main viruses infecting local garlic in Java, Indonesia

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ABSTRACT

Virus infection is one of the challenges in garlic production due to it perpetuates from one generation to the next and its infection caused huge yield reduction. There was still few information regarding virus status on Indonesian local garlic cultivars. This study was aimed to detect four major viruses infecting local garlic in Indonesia, they were members of genus *Potyvirus* (*Onion yellow dwarf virus*/OYDV, *Leek yellow stripe virus*/LYSV), and *Carlavirus* (*Garlic common latent virus*/GCLV and *Shallot latent virus*/SLV). Garlic samples were obtained from IPB University collection and field survey in Tegal and Karanganyar (Central Java Province). Dot immuno-binding assay (DIBA) was done for initial virus indexing on non-commercial and commercial cultivars. Reverse transcription polymerase chain reaction (RT-PCR) using four specific primers was done to detect virus on commercial cultivars. DIBA from leaf samples showed that virus incidence of OYDV was relatively higher (92.3 to 100%) than GCLV and SLV (84.6 to 100%) from all tested cultivars. On average, ‘Lumbu Hijau’ has the lowest level of virus titer (severity) than other cultivars. The virus incidence of both bulbil and single clover was similar (97 – 100%) while virus titer of OYDV, GCLV, and SLV on bulbil was the lowest than other propagation materials. Detection by RT-PCR from two commercial cultivars showed that ‘Lumbu Hijau’ has less virus incidence than ‘Jawa Lama’. LYSV, OYDV, GCLV were detected on both cultivars but SLV was not found. Further virus indexing using larger number of samples and involving more virus targets needs to be done.

Key words: carlavirus, dot immuno-binding assay, potyvirus, reverse transcription polymerase chain reaction

INTRODUCTION

Garlic (*Allium sativum*) together with shallot (*A. cepa* var. *aggregatum*) are two major consumed alliums in Indonesia due to their function as basic ingredients of Indonesian meals. According to Databooks (2021), the average consumption of garlic on a national scale in 2021 is 569,366 tons per year with 3.89% of consumption growth. While the demand is high and keeps increasing each year, the local farmers are struggling to meet the production target. Statistic from BPS (2021) showed that the production of garlic in 2021 by local farmers is 45,092 tons, with West Nusa Tenggara and Central Java as the highest garlic producer. This data indicated that Indonesia could only provide approximately 15.6% from the total demand, while the rest is covered by import that mostly from China (OEC, 2020). While the domestic garlic production was lower than consumption,

in 2019 Indonesian Government announced a program for self-production of garlic to reduce the import. Referring to Ministry of Agriculture Decree No. 472/Kpts/Rc.040/6/2018, some of local garlic cultivars and regions have been selected for this program to meet the target of production numbers.

Indonesia has high diversity of local garlic varieties, but only few of which were developed as commercial garlic, among others are *Lumbu Hijau*, *Lumbu Kuning*, and *Tawangmangu Baru*. *Lumbu Hijau*, *Lumbu Kuning*, *Sanur*, and *Jati Barang* are commonly grown in lowland areas. Other varieties such as *Tawangmangu Baru* and *Jawa Lama* are suitable on high land areas. All these varieties have been known to have good agronomic characters, as well as phenotypic characteristics, but they often do not do well outside their original cultivation area (Sarwadana & Gunadi, 2007). In addition, some factors such as low seed bulb quality, unsuitable climate, inappropriate cultivation technologies, pest and disease has been reported affecting the low production of local garlics (Muslihin et al., 2014).

Virus infection is one of the main problems for garlic production since its infection caused significant loss (Pérez-Moreno et al., 2014). *Potyvirus*, *Carlavirus*, and *Allexivirus* are the main genera of garlic-infecting viruses (Katis et al., 2012). In Indonesia, potyviruses

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(Onion yellow dwarf virus/OYDV, Leek yellow stripe virus/LYSV, and Shallot yellow stripe virus/SYSV) and Carlaviruses (*Garlic common latent virus*/GCLV and *Shallot latent virus*/SLV) have been detected in shallot and garlic (Harti et al., 2020; Kadwati & Hidayat, 2015; Kurniawan & Suastika, 2013). To date, *Allexivirus* has only been reported infecting *Parangkusumo* cultivar with 10% incidence (Swari et al., 2015).

Garlic is an important crop, therefore a sign of decreasing in yield and quality by virus infection may cause serious economic losses. Single infection of OYDV could reduce 48 to 65% of bulb weight; while mixed infections could reduce bulb weight from 56 to 84% (Manjunathagowda et al., 2017). Five-year study conducted by Conci et al. (2003) demonstrated that long-term infection of viruses in garlic caused significant reduction on weight and perimeter of bulbs. Information about local garlic-infecting viruses is still limited. Therefore, this research was expected to detect and measure main viruses incidence on local garlic varieties, namely *Jati Barang*, *Sanur I*, *Sanur II*, *Banjar*, and *Lumbu Kuning*. Data obtained from this research can be used as a basic information to develop disease management strategy.

MATERIALS AND METHODS

Research Site. Observation of diseases in the field and collection of samples were conducted in three locations. Samples of commercial local garlic (*Lumbu Kuning*, *Lumbu Hijau*, and *Tawangmangu Baru*) were collected directly from central production areas in Central Java Province (Tegal and Karanganyar). Samples of non-commercial local garlic germplasms (*Sanur I*, *Sanur 2*, *Jati Barang*) and commercial garlic germplasms (*Lumbu Kuning*, *Lumbu Hijau*, and *Tawangmangu*

Baru) which came from collection of Tissue Culture Laboratory, Department of Agronomy and Horticulture, IPB University were planted in the research station at Sukamantri, Bogor, West Java and then leaf samples were collected for further detection.

Sample Collection. Samples for initial virus detection were determined randomly from each block in the field using a random generator application and as many as 10% of the population from each block were collected, i.e. 43, 23, 39, 70, 40, 30 samples for *Sanur I*, *Sanur 2*, *Jati Barang*, *Lumbu Kuning*, *Lumbu Hijau*, and *Tawangmangu Baru*, respectively. Leaf from the centre of the plant was collected as samples. Samples were taken at three months after planting for virus detection using dot immuno-binding assay (DIBA). In addition, bulbils and solo garlic of *Tawangmangu Baru* and *Jawa Lama*, respectively were germinated for detection purposes due to their potential as commercial seeds. The variation of garlic propagation materials that used in this research including bulbil, solo garlic, and clove in a bulb were presented in Figure 1. For RT-PCR detection, this assay was done only for commercial garlic cultivars, *Lumbu Hijau* (2 samples) and *Tawangmangu Baru* (11 samples) that were collected from Tegal and Karanganyar, respectively.

Assessment of Virus Incidence and Titre by Dot Immuno-binding Assay (DIBA). Fresh leaf samples were put into a labelled plastic bag then stored at -80 °C or directly subjected for virus detection in the laboratory. The detection method was based on DIBA protocol as described by Wulandari et al. (2015) using commercial antibodies from DSMZ (GmbH, Germany). Virus targets for this assay were OYDV, GCLV, and SLV. The colour intensity on the membrane was used to determine virus

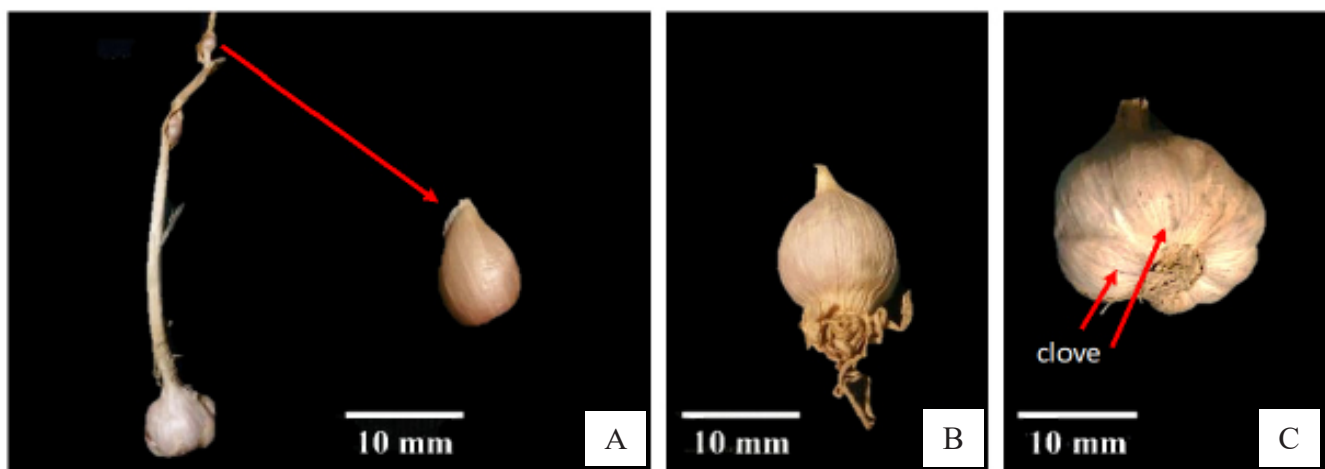


Figure 1. Variation of garlic propagation materials for samples of virus detection. A. Bulbil (right) and its position related to a bulb (left); B. Solo garlic; C. Cloves in a bulb.

titre or concentration in the sample (Kadwati & Hidayat 2015) (Table 1). The average of virus titter was then calculated using MS Excel for each garlic variety.

Reverse Transcription – Polymerase Chain Reaction (RT-PCR). Total nucleic acid extraction was done using GeneJET RNA Purification kit following the manufacturer's protocol (ThermoFisher Scientific, Waltham, USA). RT-PCR was done to detect four virus targets (LYSV, OYDV, GCLV and SLV) from two commercial local garlic cultivars, *Tawangmangu Baru* dan *Lumbu Hijau*. Amplification was conducted using one-step RT-PCR kit (ThermoFisher Scientific, Waltham, USA). Specific primer pairs for each virus target were selected according to Sumi et al. (2001) (Table 2). In one-step RT-PCR, amplification was started at 45 °C for 60 mins for cDNA synthesise then followed by one cycle of initial denaturation step at 95 °C for 1 min, then 35 cycles of denaturation (95 °C for 10 s), annealing (50 °C for 10 s), and extension (72 °C for 30 sec) and then followed by 1 cycle of final extension at 72 °C for 10 mins.

RESULTS AND DISCUSSION

Virus incidence in the field. Temanggung, Magelang, Tegal, and Karanganyar (Central Java), Malang (East

Java), Sembalun (West Nusa Tenggara), and Solok (West Sumatera) are the main garlic production areas in Indonesia based on their volume of production or areas (Titisari et al., 2019). Therefore, in this study samples were collected from Tegal and Karanganyar (Central Java Province) to represent two garlic production centres. *Lumbu Hijau*, *Lumbu Kuning*, and *Tawangmangu Baru* are the common commercial cultivars that cultivated in these areas. *Lumbu Hijau* and *Lumbu Kuning* are originally developed in East Java, while *Tawangmangu Baru* is from local Tawangmangu area in Karanganyar (Nasution et al., 2017).

Most of the garlic production area are located in the highland with elevation ~700 masl. It is a challenge to expand garlic planting area to a location in the middle-high elevation, including to provide well-adapted cultivars. Our study examined three non-commercial cultivars (*Sanur 1*, *Sanur 2*, and *Jati Barang*) grown in Sukamantri which is located in midland area, 514–582 masl with andosol type of soil, pH between 5.0 until 5.6 and climate type A1 with the average of rainfall 500 mm from February to June 2018 and temperature around 19 to 27 °C (BMKG, 2018). The condition is unfavourable in Sukamantri for growing garlic. According to Permana (2006) and Setiawati et al. (2007) ideal growth of garlic requires dry climate with humidity around 60 to 70%; temperature around 15 to 20 °C; rainfall of 100–200 mm/

Table 1. Description of titter index based on colour intensity





Titer index	Colour intensity	Description
0		Negative reaction (-)
1		Weak reaction
2		Moderate reaction
3		Strong reaction

Table 2. List of specific primers for amplification of OYDV, GCLV, dan SLV

Target	Primer	Primer sequences (5′– 3′)	Product size (bp)	Reference
LYSV	P-RT3	AAGAGTCAACACTTGGTTTG	191	Sumi et al. (2001)
	P-RT4	GGTCTCAATCCTAGCTAGTC		
OYDV	OG-RT1	GAAGCGCACATGCAAATGAAG	290	
	OG-RT2	CGCCACAACCTAGTGGTACAC		
GCLV	GC-RT1	AATGGGTGTTCTAGGAGTGC	306	
	GC-RT2	TTAAACCTTAGTCAAGCTATTC		
SLV	GS-RT1	TATGCTCGAGCTCGTAGAGC	170	
	GS-RT2	GGGTTTCACATTGTTACACC		

month; well-drained soil with pH level range from 5 to 5.7; and plenty of sunshine. Among all cultivars planted in Sukamantri, *Sanur 1* had the strongest adaptability based on their agronomic performances (data was not shown). Further experiment is required to improve the performance of potential cultivars for the middle high growing areas.

The symptoms caused by virus infection varied among garlic varieties, in Sukamantri was dominated by yellow mosaic and yellow stripe (Figure 2), while in Tegal and Tawangmangu were yellow stripes, green mosaic, and yellow mosaic. In the field, garlic was uniformly infected by *Potyvirus*, *Carlavirus*, and *Allexiviruses* which produced chlorotic stripe on the leaves and reduction on plant height and bulb size (Cremer et al., 2021).

The result showed that virus incidence in Sukamantri was relatively high with the range from 84.6 (33/39) to 100% (23/23–43/43) (Figure 3) based on virus detection from leaves. The highest and lowest average of virus incidences were found on *Sanur 1* and *Jati Barang*, respectively. Similarly, the incidence from commercial fields (*Lumbu Hijau* and *Tawangmangu Baru*) was also high, ranging from 90% (36/40) to 100% (40/40) (Figure 3). Among the three targeted viruses, OYDV was more frequently detected than GCLV and SLV.

The highest and lowest average of virus titer were found in *Tawangmangu Baru* and *Jati Barang*, respectively (Figure 4). Overall, virus titer in garlic plants was considered varied from weak to moderate, with the range from 0.95 to 1.89 except for intensity of GCLV infection in *Tawangmangu Baru* which showed



Figure 2. Symptoms of virus infection in the field. A. Yellow stripe; B. Yellow mosaic.

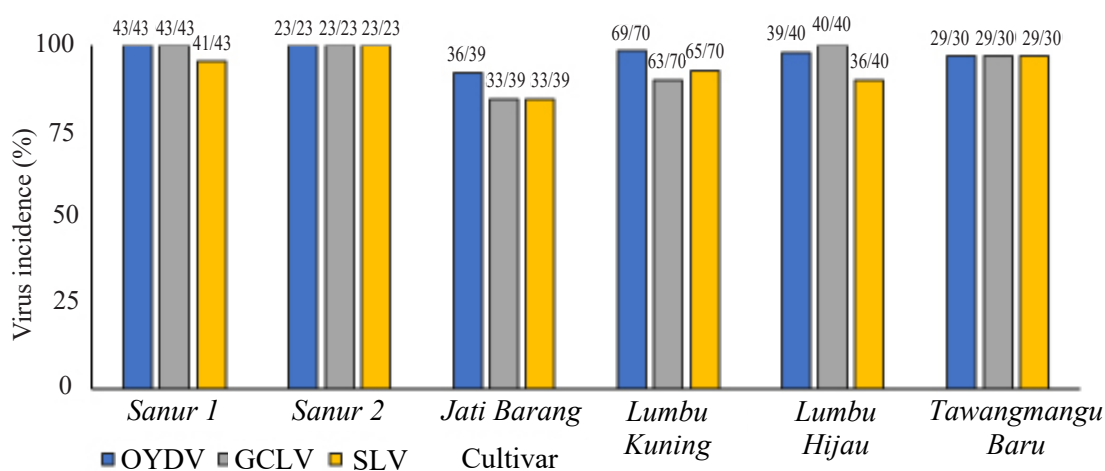


Figure 3. Incidence of OYDV, GCLV, and SLV from leaves of non-commercial (*Sanur 1*, *Sanur 2*, and *Jati Barang*) and commercial (*Lumbu Kuning*, *Lumbu Hijau*, and *Tawangmangu Baru*) local garlic cultivars based on detection using DIBA method.

strong reaction (2.05). Interestingly, *Lumbu Hijau* as commercial cultivar has low to moderate titer intensity and less infection than other cultivars except for SLV infection in *Jati Barang*.

Detection of garlic viruses on propagative materials.

All propagative materials of *Tawangmangu Baru* and *Jawa Lama* were infected by OYDV, GCLV, and SLV (Figure 5). The incidence of virus infection on both bulbil and solo garlic were similar, ranging from 97% (47/50)–100% (50/50). Meanwhile, virus infection in clove from both cultivars showed lower incidence than bulbil and solo bulb, especially for SLV infection in

Tawangmangu Baru (72%, 36/50). In general, titer of OYDV, GCLV, and SLV were varied on both cultivars and propagation materials (Figure 6). The titer of OYDV and GCLV were moderate to strong while SLV titer was moderate in all samples. We also noted that titer of OYDV, GCLV, and SLV on bulbil was the lowest than other propagation materials even though the virus incidence was not quite different. Previous study indicated that bulbil might become a good planting material due to its pathogens (bacteria and fungi) free and less virus infection (Bushal et al., 2021; Fan et al., 2017; Shemesh-Mayer et al., 2022; Sulastiningsih et al., 2020). To date, only *Potyvirus* that was found on

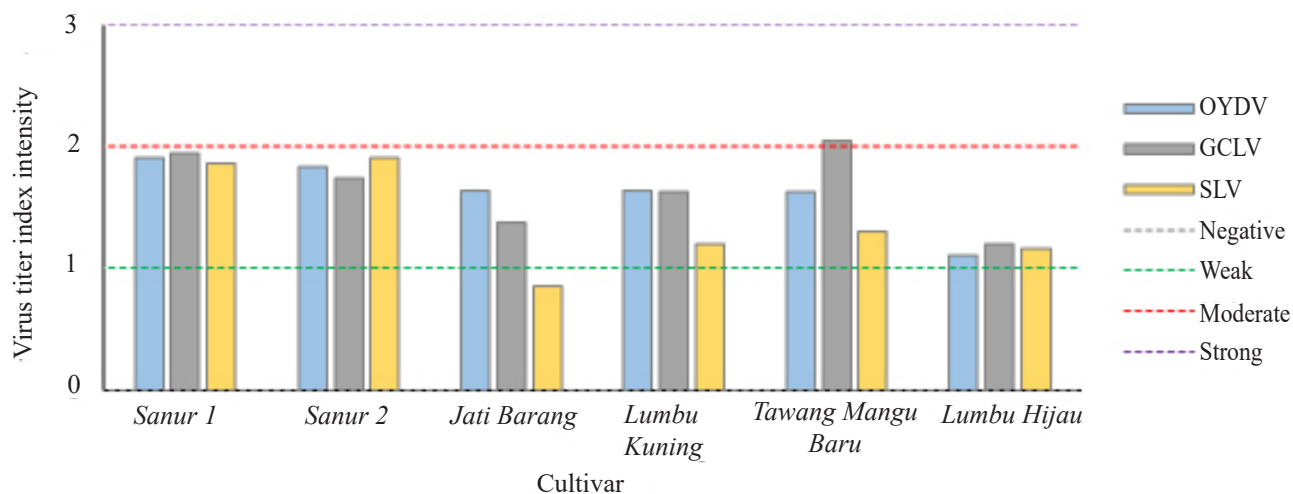


Figure 4. Index intensity of virus titer for OYDV, GCLV, and SLV detected from leaves of non-commercial (*Sanur 1*, *Sanur 2*, and *Jati Barang*) and commercial (*Lumbu Kuning*, *Lumbu Hijau* and *Tawangmangu Baru*) local garlic cultivars based on DIBA method. The colour intensity was presented using score 0–3, i.e., score 0= negative; 1= weak; 2= moderate; 3= strong.

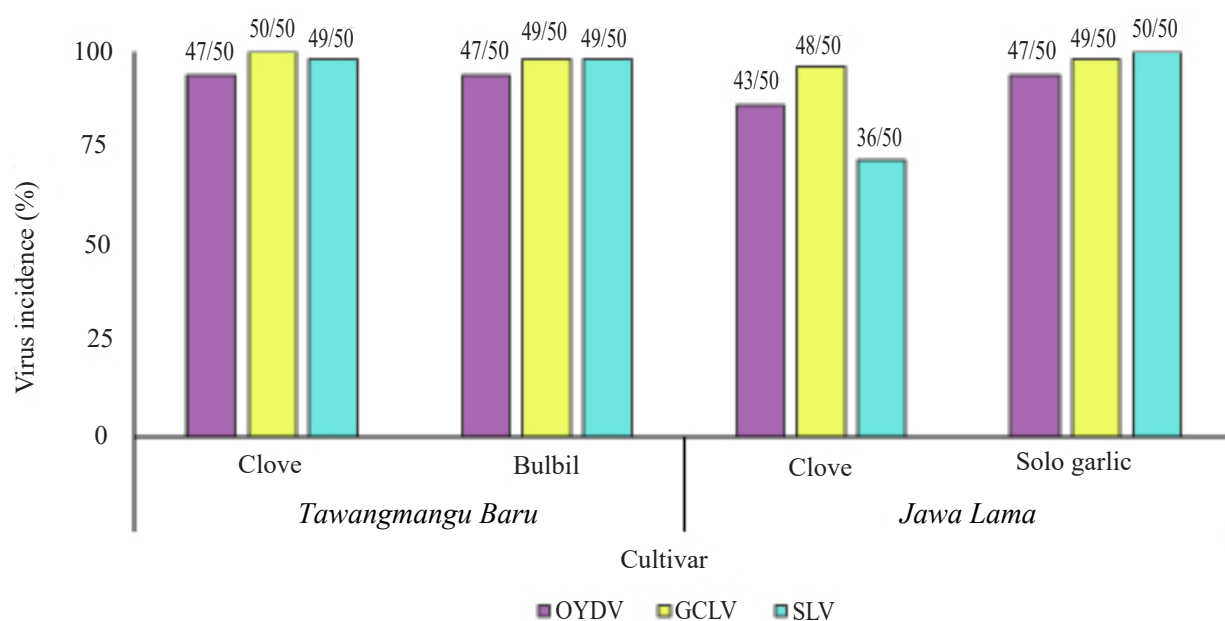


Figure 5. Incidence of OYDV, GCLV, and SLV from clove, bulbil, and solo garlic based on detection using DIBA method.

true-seeds garlic (Shemesh-Mayer et al., 2022). Recent study suggested that the presence of potyvirus on the young developing flowers occurred either through cell-to-cell movement or phloem tissue (Shemesh-Mayer et al., 2022).

The important finding of our study was the evidence of virus infection both in the field and propagative materials. The data of virus indexing also highlighted the incidence of virus mix infection on all propagative materials. It has been reported by Klukáčková et al. (2007) that mix infection, for instance OYDV and LYSV, GCLV and SLV are commonly occurred in garlic plants. Virus mix infection on individual plant plays an important role on each virus pathogenicity and disease severity (Perotto et al., 2010; Takaichi et al., 1998). The data on the virus incidence implied that all propagative materials (except pollen) have been already infected by potyviruses and may contribute as primary inoculum in the field (Shemesh-Mayer et al., 2022). Therefore, it is highly recommended to use virus-free bulbs as initial planting material despite

the difficulties to provide commercial virus-free planting material in large numbers. The use of virus-free planting material also can delay the virus infection in the field and increase the bulb weight (Conci et al., 2003; Filho et al., 2006; Salomon, 2002). As for the alternative, Pérez-Moreno et al. (2014) suggested to use good quality seed bulb from garlic elite lines which will produce higher yield even though they were not virus-free.

Virus confirmation by RT-PCR. RT-PCR detection from leaf samples confirmed the infection of three virus targets, with the highest frequency was LYSV, OYDV, GCLV, sequentially (Table 3). All leaf samples were positively infected by three virus targets, except OYDV on samples from Tegal and one sample from Tawangmangu. Interestingly, there was no SLV infection on both leaf and clove samples from Tegal but we detected SLV from IPB's germplasm collection with the same cultivar (cultivar *Lumbu Hijau*). This might happen because the distribution of viruses on different cloves within one bulb was uneven (Godena et al., 2020). The

Table 3. Incidence of main viruses infecting local garlic in Tawangmangu and Tegal based on detection using RT-PCR method

Virus target	Origin of samples	
	Tawangmangu	Tegal
LYSV	11/11*	2/2
OYDV	6/11	2/2
GCLV	1/11	2/2
SLV	0/11	0/2

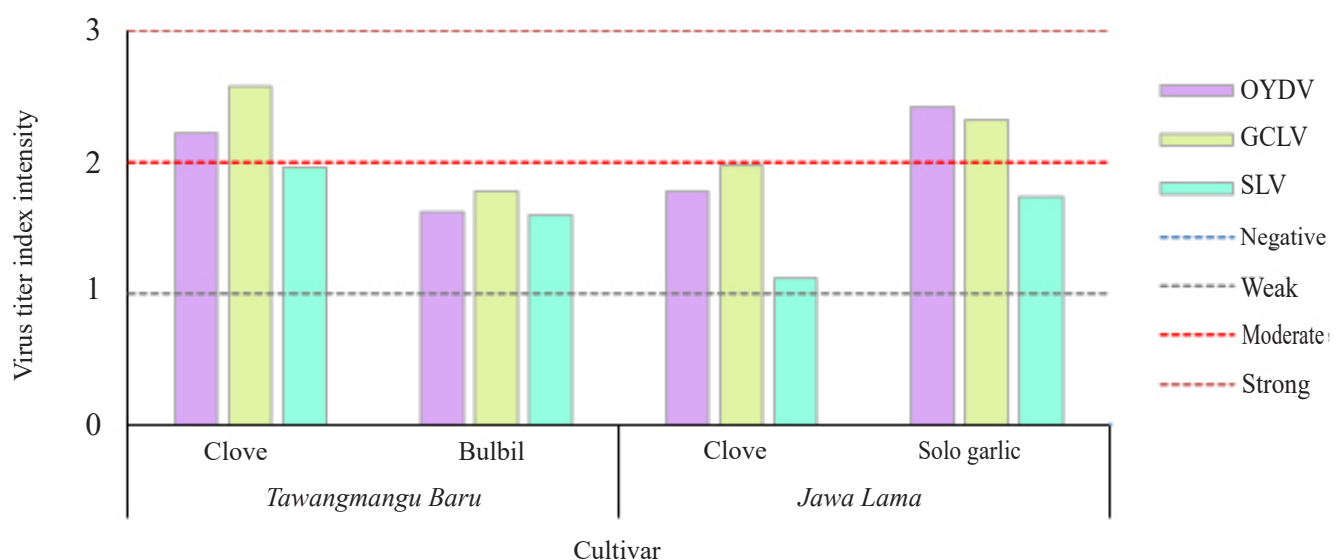


Figure 6. Index intensity of virus titer for OYDV, GCLV, and SLV detected from clove, bulbils, and solo garlic based on DIBA methods. The colour intensity was presented in score range 0–3, i.e., score 0= negative; 1= weak; 2= moderate; and 3= strong.

second possibility is the different age of plants when the sample was taken. The variation of virus accumulation on different plants relied on plant stage (Conci et al., 2003; Godena et al., 2020).

There are eleven main viruses infecting garlic worldwide that has been reported, which belong to *Potyvirus*, *Carlavirus*, and *Allexivirus* members (Katis et al., 2012). Further study to detect more virus target in Indonesian garlic can be done using advanced detection techniques such as high throughput sequencing. Comprehensive knowledge about the characters of viruses infecting garlic will help the development of disease management strategy.

CONCLUSION

High incidence of four major garlic viruses (LYSV, OYDV, GCLV and SLV) has been detected from garlic commercial production areas in Tegal and Karanganyar, Central Java. In addition, three major garlic viruses (OYDV, GCLV, and SLV) were detected using DIBA method on non-commercial local garlic germplasm in research station in Bogor, West Java. Similarly, infestation of OYDV, GCLV and SLV was detected from all planting materials. This information should be taken into consideration because virus infection has the potential to cause significant garlic yield loss.

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AUTHORS' CONTRIBUTIONS

SHH designed experiment, interpreted data, wrote and edited manuscript. ME and RY carried out sample collection, field experiment, and serological test. DD designed experiment, provided germplasm collection, and analysed data. SN performed data analysis and molecular test, wrote manuscript.

COMPETING INTERESTS

The authors declare no conflict of interest.

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