Distribution of Major Viruses on Shallot in Indonesia

Heri Harti[1](✉), Sobir Sobir[1], Suryo Wiyono[1], and Sri Hendrastuti Hidayat[2]

[1] Center for Tropical Horticulture Studies, IPB University, Bogor, Indonesia
heri.harti16@gmail.com

[2] Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia

Abstract. High infestation of viruses on shallot's bulb has been reported in Indonesia, although little is known on the effect of virus infection on shallot productivity. The use of virus-free seeds is assumed to be the key factor to improve productivity. The study was conducted to detect the main viruses infecting shallots and obtain data on their spread in Indonesia. Research activities included survey to several production centers of shallots (Brebes, Probolinggo, Solok, Bima and Enrekang) to observe the incidence of disease and collect samples for identification of viruses in the laboratory. The main symptoms found in the field involved flat leaves with yellow striped leaves in the center, green stripes, yellow stripes and wrinkled. Four main viruses, namely OYDV, SYSV, GarCLV and SLV were successfully detected by the dot immunobinding assay (DIBA) method and were found in Brebes, Probolinggo, Solok, and Enrekang. Based on observations of symptoms in the field it was known that the highest incidence of viral diseases is in Brebes (70.81%) while the lowest was in Bima (25.17%). Factors influencing the incidence of the virus included planted varieties, altitude, the origin of the seeds, and application of manure.

Keywords: Disease incidence · Dot immunobinding assay · Origin of seeds · Production center · Survey

1 Introduction

Shallot (*Allium cepa* var. *Ascalonicum*) is one of important horticultural comodditties in Indonesia due to its significant contribution to the regional economic. Shallot production in Indonesia reached 1,446,860 tons in 2016 [1]; with economic value reaches up to IDR 14.47 trillion/year. Six provinces, namely Central Java, East Java, West Java, West Nusa Tenggara, West Sumatra and South Sulawesi are the main shallot-producing areas with a contribution of 95.13% to the average Indonesian shallot production. Brebes, Bima, Solok, and Enrekang districts are the largest production centers in Central Java, West Nusa Tenggara, West Sumatra, and South Sulawesi, respectively, with 72.39%, 75.80%, 95.27%, and 82.76% of total shallot production in each province. Probolinggo Regency is the second largest production center in East Java after Nganjuk Regency, contributing 19.46% of the total shallot production in East Java.

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Shallot propagation using bulb is a common practice in Indonesia. It potentially becomes a problem when farmers did not select the best bulb for planting material. Some pathogens, especially viruses, have been known to be bulb-borne and accumulated from one generation to another through bulb [2]. Three genera of viruses that has been reported to heavily infect shallot are Carlavirus (*Shallot latent virus*/SLV and *Garlic common latent virus*/GarCLV), Potyvirus (*Onion yellow dwarf virus*/OYDV, *Shallot yellow stripe virus*/SYSV, and *Leek yellow stripe virus*/LYSV), and Allexivirus (*Garlic virus B*/GV-B, *Garlic virus C*/GV-C, and *Garlic virus D*/GV-D) [2, 3]. In fact, high infestation of viruses on shallot's bulb has been reported in Indonesia [4–8]. Infection of Potyvirus, Carlavirus, and Allexivirus was detected from shallot samples collected from Brebes, Central Java and Cirebon, West Java [6, 9]. Furthermore, Wulandari and Hidayat [8] reported wider spread of OYDV, SLV, and GarCLV on shallot in Indonesia. The study was conducted to detect the main viruses infecting shallots and obtain data on their spread in Indonesia.

2 Material and Methods

2.1 Survey and Sample Collection

The study was conducted from September 2016 to October 2017. The survey was conducted respectively on 10 shallot fields in Central Java (Brebes), East Java (Probolinggo), West Sumatra (Solok), West Nusa Tenggara (Bima), and South Sulawesi (Enrekang). Each field is divided into 10 blocks, respectively consisting of 10 clumps of shallot plants. Then observed the incidence of disease based on symptoms in the field. In addition to observing symptoms, samples of shallot bulbs were also taken from each survey location. A total of 10 clumps of shallots from each block were mixed, so that in 1 plant block there was 1 composite sample. Overall, 100 composite samples were obtained from 10 shallot fields in each production center. The bulbs samples were put into nets, labeled, and then brought to the laboratory. In the laboratory, shallots bulbs are grown for detection and identification viruses. In addition to taking bulbs samples, at the time of doing the survey, interviews with farmers were also conducted and observations of field conditions, especially related to cultivation factors that affect crop yields.

2.2 Virus Detection Using the DIBA Method

Bulbs collected from the field were grown in the laboratory, then leaf samples were taken when the plants were 30 days after planting. Leaf samples were used as material for virus detection. Virus detection was conducted following method described by Asniwita et al. [10] using antibodies for OYDV, GarCLV, and SLV.

Leaf samples were weighed 0.1 g, then grounded in Tris Buffer Saline (TBS, *Tris*-HCl 0.02 M dan NaCl 0.15 M, pH 7.5) 1:10 (w/v). Plant sap was spotted onto nitrocellulose membrane about 2 μL. The membrane was air dried, then it was submerged into 2% non-fat milk and TBST buffer (Tris buffer saline with Triton X-100) solution. The membranes were incubated at room temperature and shaked at 50 rpm for 60 min. Membranes were then washed 5 times with H2O, each washing process were shaken at 100 rpm for 5 min.

After washing, the membranes were soaked into 2.5 μL first antibody solution (with 2% non-fat milk-TBST), then incubated at 4 °C overnight. After washing 5 times for 5 min using TBST, the membranes were soaked on 2.5 μL conjugate antibody (with 2% non-fat milk-TBST), then incubated for 60 min. The membranes were soaked on 10 mL AP-buffer (Tris-HCl 0.1 M, NaCl 0.1 M, MgCl2 5 mM, H2O) containing 1 tablet of nitro blue tetrazolium (NBT) and bromo chloro indolil phosphate (BCIP). Positive reaction was qualitatively indicated by appearance of purple colour in nitrocelullose membrane on the spotted area. Reaction was stopped by soaking the membranes onto H2O.

2.3 Data Observation and Analysis

Based on the observation of symptoms in the field and the results of DIBA, the incidence of virus disease was calculated using formula:

$$\text{Disease incidence} = \frac{\text{number of samples infected}}{\text{number of samples observed}} \times 100\%$$

The number of infected plants was determined based on the results of virus detection using the DIBA method. Analysis of data variance was conducted using the PKBT-STAT 2.1 program. Data analysis of the factors that influence the incidence of the virus was conducted using the t test in the excel program.

3 Results

3.1 General Condition of Shallot Farm in Production Center

Brebes, Probolinggo and Bima are lowland areas, while the Enrekang is a mediumland area, and Solok is a highland area. The conditions for farming and cultivating shallots was condusted by farmers in each area are varied (Table 1).

Shallots generally grown as monocultures in all production centers. However, intercropping of shallots with other horticultural crops such as chili, eggplant and caisin was also found. Shallot cultivation in Brebes and Probolinggo is done in paddy fields, while in Enrekang, Bima and Solok cultivation is done on dry land. The planting of shallots in all production centers knows no seasons. The growing season in Brebes is at the end of the rainy season that is May to June and August to September, with the main planting season in June. However, beside of May/June, farmers in Brebes still plant shallots in other months. Similar to Brebes, planting in Probolinggo is done all the year with the planting season at the end of the rainy season, but the main harvest season in Probolinggo is from June to August. Most of the shallot farmers in Brebes and Probolinggo apply a cropping pattern of 3 times each year, namely rice-shallot I-shallot II-shallot III.

Solok, Bima and Enrekang are off season shallot production areas. Shallot cultivation in this area is mostly done in the rainy season, from November to April. In Solok shallot was harvested on January to February, while in Bima harvest was done on June to July and September to October, and in Enrekang harvest was done in January.

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Table 1. General conditions of shallots cultivation and cultivation techniques in some production centers

Variable	Production center				
	Brebes	Probolinggo	Bima	Solok	Enrekang
Altitude (above the sea level)	7–10 m	4–47 m	15–25 m	1496–1556 m	426–883 m
Variety	Bima Curut	BirucLancur	Super Philips	Alahan Panjang	Kapur (Katumi)
Plant age (DAP)	40–42	30–35	30–35	35–40	31–40
Origin of seeds	self-produce	self-produce	self-produce	self-produce	trader
The spacing (cm)	15 × 20	18 × 20	Tidak tentu	20 × 20	20 × 20
The use of manure	no	not enough	not enough	enough	not enough
Main weed	no	grass	main weed	main weed	grass
Main pests	*Spodoptera.exigua*	*S. exigua*	*S. exigua*	*S.exigua* *Lyriomiza* sp.	*S. exigua*
Main disease	Fusarium wilt	Fusarium wilt	Fusarium wilt	Not found	Fusarium wilt
Pest control	pesticide	pesticide	pesticide	pesticide	pesticide

DAP = Days After Planting

Shallot cultivation in Brebes is almost the same as Probolinggo, namely using ditches for irrigation between beds. Shallot cultivation in Bima does not use ditches for irrigation, because it only relies on rainfall. Likewise in Enrekang, irrigation system in the form of sprinkler irrigation are self-supported by farmers. In contrast to other areas, the cultivation of shallots in Solok uses plastic mulch and no special irrigation system. Shallot plantations in Probolinggo and Enrekang and Bima were not using enough organic matter, while in Brebes it is almost non-existent. But farmer in Solok used enough organic materials.

The main pest that caused heavy damage was *S. exigua*, while the main disease was fusarium wilt or "moler", which is caused by *F. oxysporum* fsp *cepae*. There were no virus vectors (aphids) found in the fields. Control of plant pest and disease is done using

Fig. 1. Variations of symptoms of virus infection on shallots: (a) wrinkled; (b) flat leaves with light yellow stripes in the middle; (c) green striped leaves; (d) yellow striped leaves

intensive application of pesticides in all production centers. In general, shallot farmers are not familiar with the symptoms of virus infection and its impact on decreased production.

3.2 Symptoms of Virus Infection in the Field

Symptoms of virus infection in shallots found in the field varied, namely flat leaves with light yellow stripes in the middle, green stripes, yellow stripes and wrinkled. In addition to a single symptom, a mixture of various symptoms was found (Fig. 1). Symptoms of yellow mosaic accompanied by yellow vertical stripes and green stripes were previously reported by Kadwati et al. [6] and Gunaeni et al. [5].

All the above symptoms were found in Brebes and Probolinggo. In Bima and Enrekang there were no symptoms of wrinkled leaves, while in Solok only flat and wrinkled leaves were found. In one plant can be found more than one symptom of virus infection.

3.3 Disease Incidence and Virus Infections in the Field

Based on observations of symptoms in the field, it is known that in all survey areas virus infections have been found in shallots (Table 2). The highest incidence of virus disease is in Brebes (70.81%) and the lowest is in Bima (25.17%).

Virus detection from field samples using the DIBA method confirmed OYDV, SYSV, SLV and GarCLV infections. Virus presence could be detected in both symptomatic and healthy-looking shallot. The DIBA results of Enrekang sample showed 99.0% of the samples were infected, while the Brebes and Probolinggo samples were respectively 95.5% and 91.5% infected. Solok and Bima samples were respectively only 59.0% and 54.0% infected (Table 2). The difference between the results of incidence observation in the field and the results of detection in the laboratory is caused by the character of the virus infection that can cause latent symptoms. Klukackova et al. [11] reported that SLV and GarCLV often did not show clear visual symptoms.

The DIBA results showed that the highest virus incidence occurred in Enrekang with positive samples for OYDV, GarCLV, SLV, and SYSV, respectively 93.5%, 80.5%, 80.0% and 93.0%. In Brebes, the number of positive samples for OYDV and SLV reached

Table 2. The incidence of virus disease on shallots is based on observation of symptoms in the field and detection by the DIBA method

Location	Disease incidence (%) based on	
	symptoms in the field	detection by the DIBA
Brebes	70.81	95.50
Probolinggo	36.84	91.50
Bima	25.17	54.00
Solok	27.68	59.00
Enrekang	40.71	99.00

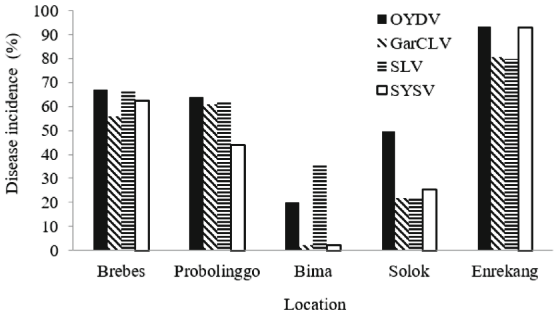

Fig. 2. The incidence of the main virus in samples of shallot bulbs from some production centers based on the results of detection by the DIBA method

67.0%, followed by SYSV (62.5%) and GarCLV (56.0%); while in Probolinggo the most OYDV positive samples were found (64.0%), followed by SLV (61.5%) and GarCLV (61.0%), and SYSV (44.0%). In Solok, the most positive samples were OYDV (49.5%), followed by SYSV (25.5%), GarCLV (22%) and SLV (21.5%). In Bima, samples of OYDV (20%), GarCLV (2%), SLV (36%) and SYSV (2%) were found (Fig. 2). In each sample area the leaves were not only infected with one type of virus but infected with more than 1 virus.

According to Gunaeni et al. [5], among 20 shallot samples taken from West Java and Central Java, SYSV was detected in 95% of them. Reference [8] reported that seed bulbs samples from Cirebon, Brebes, Majalengka, Kuningan and Bandung tested positive for OYDV (75.57%), SLV (41.95%), and GarCLV (10.0%). The study results of [7] shown that some shallot varieties in Bantul were positively infected with Carlavirus and Potyvirus. Kadwati et al. [6] succeeded in detecting GarCLV, SLV, and Potyvirus from shallot samples from West Java (Bandung, Bogor, and Cirebon) and Central Java (Brebes) and Yogyakarta (Bantul). The average percentage of virus infections in the field ranged from 11.22% to 14.29%, while in bulbs samples it ranged from 9.18% to 13.27%.

Table 3. DIBA score of virus detection results from shallot leaf samples

Location	Virus infection score with specific antibodies			
	OYDV	GarCLV	SLV	SYSV
Brebes	0.77	0.56	0.86	0.67
Probolinggo	1.22	1.135	0.78	0.44
Bima	0.00	0.00	0.58	0.04
Solok	0.66	0.22	0.22	0.26
Enrekang	1.26	1.38	1.55	1.47

The antiserum reaction on the DIBA results showed a different intensity of color change. The intensity of this color change reflects the virus titer in the plant tissue. Virus titers in plant tissues were determined by scoring the intensity of color change on the DIBA results (Table 3). The higher the DIBA score, the higher the virus titer in the plant tissue tested.

The color intensity score on the membrane showed that samples from Enrekang had the highest score (1.26–1.55) for all tested viruses, followed by samples from Probolinggo (0.44–1.20) and Brebes (0.67–0.86). Meanwhile, the Bima sample had the lowest score (0.00–0.58). This score is in appropriate with the DIBA results which detected that virus infection in samples from Enrekang was the highest, reaching 99%. Although the incidence of virus disease is high, the virus titer contained in plant tissue is still relatively low.

3.4 Factors Affecting Disease Incidences

Environmental factors where shallots grow, varieties used, and agricultural practices applied by farmers can affect virus incidence on shallot. The results of statistical analysis showed that the types of varieties used had a significantly different effect on virus incidence (in the field and DIBA results) (Table 4). The effect of variety to the virus incidence is related to the resistance of the variety to virus infection. Based on observations of symptoms in the field, the Bima Curut variety from Brebes showed the highest virus incidence, while the Superphillip variety was the lowest. The highest virus incidence based on DIBA results was found in the Katumi variety and the lowest in the Superphillip variety.

Environmental and agricultural practices factor that affect the virus incidence were altitude, origin of seeds and application of manur (kg/ha). The results of t-test on the effect of altitude, origin of seeds and the number of applications of manure/ha on the incidence of the virus can be seen in (Table 5). Altitude has a significant effect on virus incidence based on observations of symptoms in the field, while based on DIBA results it has no significant effect. The higher the location, the lower the incidence of the virus. The origin of the seed had a significant effect on the incidence of the virus based on the DIBA results. The incidence of local seed virus is lower than seedlings obtained from other areas.

Table 4. Effect of variety on virus incidence

Variety	Average incidence of virus (%)	
	On the field	DIBA result
Bima Curut	76.3a	65.05b
Biru lancor	38.9b	53.11b
Superphillip	26.5c	15.15c
Solok Sakato	26.2c	26.21c
Katumi	43.2b	99.24a

Note: numbers followed by different letters in the same column are significantly different

Table 5. T value of environmental and agricultural practices effect on the incidence of virus

Parameter	T value	
	On the field	DIBA result
Altitude (m above sea level)	0.0386	0.1089
The origin of seed	0.2168	0.0386
The use of manure (kg/ha)	0.0087	0.0436

4 Discussion

The result of this study indicates that shallots in Brebes, Probolinggo, Solok, Bima and Enrekang have been infected with viruses with varied symptoms and incidences of disease. Mohammad et al. [12] showed that OYDV, SLV, LYSV and GarCLV infect garlic plants in South Syria with a disease incidence of 15–90%. The symptom in garlic is a yellow mosaic with yellow vertical stripes and green stripes previously reported by Kadwati et al. [6], Wulandari et al. [8], and Gunaeni et al. [5]. Yousif et al. [13] reported that the symptoms of OYDV infection in Sudan are the leaves were semi flat, flaccid, and curled with yellow green streaks altering with deep green background of the leaf in a form of bands. The whole plant was stunted with drooping leaves. Later, it produced a short twisted and very thin flower stalk, umbels were very small. OYDV infection symptoms, will only get worse when the shallot was infected by SLV and SYSV. Symptoms did not show up as the plant gets older [14].

In general, infecting virus cannot be distinguished by symptoms. In one plant alone, it could be infected by multiple virus, but will shows several symptoms. Klukackova [11] reported that SLV and GarCLV infections often do not show clear visual symptoms. To determine which viruses, infect shallots in the field, detection is done in the laboratory using accurate and sensitive methods, such as DIBA. The DIBA results showed that four major viruses were found in all locations. Differences in virus incidence depends on the type of variety grown, agricultural practices, the susceptibility of a plant to viruses or insect vectors [15], environmental conditions such as temperature, humidity, and rainfall

[16], the type or strain of the virus in an area, and the presence of mixed infections with other viruses [17].

Analysis of variance and t-test showed that virus incidence was influenced by several factors, namely the variety used, altitude, origin of seeds, and application of manure (kg/ha). The virus incidence was higher in the lowland, because of hotter air temperature, which caused the symptoms of virus infection to be more expressed. Virus infection of local seedlings was lower than the seeds obtained from other areas. This is presumably in due to the seeds obtained from other areas are sold by traders, which were the low-quality consumption bulb. According to Putrasamedja [18], seeds from consumption bulbs commonly used by farmers has lower quality, because it's not produced with a selection process so that the possibility it being infected with virus is greater. Manure application affects plant fertility. Sufficient application of manure causes plants to be more vigor and healthy, thus indirectly causing a lower virus incidence. In addition to the above factors, the increase in virus infections in the field can be caused by the presence of pathogenic inoculum at the beginning of plant growth [19]. Viruses will be spread by vectors to other plants. Ahmed and Elhassan [20] reported that the high rate of OYDV infection in Khartoum and riverside of Nile was mainly due to the planting time in the area, i.e., late winter coincided with the abundance of aphid vectors whose activity was very high around the source of infection and infected seed bulbs. Meanwhile the low virus infection on bulbs collected in autumn growing in Gezira and Kassala State could be attributed to the absence of a major source of infection and/or limited vector activity, resulting in very limited spread of virus by vectors.

The high incidence of the virus in Enrekang, Brebes and Probolinggo is thought to be caused by the origin of the seeds. The Katumi variety seed bulbs used in Enrekang were purchased from traders in Nganjuk, while in Brebes and Probolinggo they used Bima and Biru Lancur variety which were produced by the farmers themselves. In Solok and Bima, the incidence of the virus was lower, because the sources of seeds used were Solok Sakato and Superphillip, where these varieties were local varieties that had not been widely circulated.

The incidence of virus on shallots in the field is caused by farmers using virus-infected bulbs as plant propagation material. The shallots virus is a bulbs-borne pathogen. The spread of the virus on shallots in various regions in Indonesia is related to the spread of plant propagation materials, namely bulbs that are infected with the virus. Bulbs infected with the virus were the main source of inoculum in the field and were spread by vectors to other crops. Most farmers use seeds from bulbs from previous harvests or buy from farmers in other areas from previous growing season. The quality of the shallot seed bulbs used by farmers with this system certainly does not pass the seed health check process, so the chance of carrying the virus and spreading the virus along with the spread of seeds between regions is getting bigger. To avoid the distribution of the virus, it is best to avoid the circulation of seeds from areas with high infection rates to areas with low infection rates.

5 Conclusion

Four main shallot viruses, that is OYDV, SYSV, GarCLV and SLV, were found in Brebes, Probolinggo, Bima, Solok, and Enrekang with an incidence ranging from 54 to 99%.

The type of virus with the highest infection rate was OYDV from the Potyvirus group, with the highest incidence of OYDV (93.5%) found in Enrekang. The Virus incidence in various areas of shallot production centers in Indonesia is influenced by variety, altitude, origin of seeds, and application of manure.

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