Variations of *Garlic Common Latent Virus* and *Shallot Latent Virus Concentration* on Shallot and Garlic

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Variations of Garlic Common Latent Virus and Shallot Latent Virus Concentration on Shallot and Garlic

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Abstract. Infection of Garlic common latent virus (GCLV) and Shallot latent virus (SLV) on shallot and garlic has been reported in Indonesia. Both viruses are known to infect the plants in the field in high incidence and can be transmitted through the bulbs. A research was conducted to determine the potency of infected bulbs as source of disease inoculum by measuring virus titer throughout bulb growing stadia. Two kinds of shallot’s and garlic’s bulbs was used, i.e. shallot seed bulb (cv. Bima curut), garlic seed bulb (cv. Sembalun), consumption shallot and garlic bulb (unknown cultivar). The experiment was conducted using completely randomized design. Ten samples with three repetition of each stadia, i.e. adventitious shoot, shoot, leaf on 7 days after planting (DAP), and 14 DAP were analyzed by enzyme-linked immunosorbent assay using specific antisera to GCLV and SLV. Infection of GCLV and SLV was found higher on garlic than on shallot. Both viruses were detected in high incidence from adventitious shoot and 14 DAP-foliar leaves. Virus titer is higher on consumption bulbs than those on seed bulbs for both shallot and garlic. This study indicated that infected bulbs is very potential as the primary source of disease in the field.

Keywords: carlaviruses, enzyme-linked immunosorbent assay (ELISA), primary inoculum, virus titer

1. Introduction

Shallot (Allium cepa var. aggregatum) and garlic (A. sativum) are important crops in Indonesia. Shallot is cultivated in many places in Indonesia, with the main production area are Cirebon (West Java), Brebes (Central Java), Bantul (Yogyakarta), and Nganjuk (East Java). Productivity of shallot were fluctuated from 2012 to 2016, i.e. 9.69 tons/ha, 10.22 tons/ha, 10.22 tons/ha, 10.06 tons/ha, and 9.66 tons/ha, respectively [1]. In contrast, garlic cultivation is getting less in Indonesia and its productivity is more fluctuated than shallot from 2011 to 2015, i.e. 14.749 tons, 17.638 tons, 15.776 tons, 16.894 tons, and 20.293 tons, respectively [2].

Pests and diseases is a big concern on shallot and garlic cultivation. Viruses are a particular problem in bulb crops such as shallot and garlic, since vegetative propagation leads to accumulation and dissemination of viruses in planting material. The most common viruses infecting shallot and garlic are Garlic common latent virus (GCLV) and Shallot latent virus (SLV), members of genus Carlavirus; Onion yellow dwarf virus (OYDV), Shallot yellow stripe virus (SYSV), and Leek yellow stripe virus (LYSV), members of genus Potyvirus, and mite-borne filamentous virus (MbFV), member of genus Allexivirus [3]. Infection of OYDV, LYSV, and SLV has been reported on shallot in...
Indonesia [4,5]. Moreover, Kadwati and Hidayat [6] reported GCLV and SLV infections were found on shallot and garlic from Yogyakarta (Bantul), Central Java (Brebes), and West Java (Bandung, Bogor, and Cirebon). Mixed virus infection on shallot and garlic was frequently occurred.

Viral disease management should follow integrated disease management concept, involving several control methods, i.e. control insect vector, remove inoculum sources, avoid new infection. The use of healthy and virus free propagation material is a good disease management strategy for shallot and garlic, because infected bulb may become the primary inoculum sources in the field. The potency of bulbs as virus source can be predicted by measuring the virus titer on plant tissue.

Many methods have been developed for detection and identification of plant viruses using serology and molecular methods. The most common used serology methods are enzyme-linked immunosorbent assay (ELISA) and dot immunobinding assay (DIBA), while the most common used molecular method is polymerase chain reaction (PCR) [7]. ELISA is relatively easy to perform, have high sensitivity and specificity, and the result can be analyzed quantitatively [8]. Quantitative measurement of absorbance value of ELISA can be used to estimate the virus titer (concentration) in plant tissue [9]. This study was conducted to determine the virus titer from shallot and garlic tissue from several plant stadia.

2. Materials and methods

2.1. Growing shallot and garlic seed bulbs
Shallot and garlic seed bulbs were obtained from seed grower and local market (consumption). Shallot seed bulbs were obtained from Brebes, Central Java i.e. cv. Bima Curut; whereas garlic seed bulbs were obtained from Sembalun, West Nusa Tenggara, i.e. cv. Sembalun. Bulbs of shallot and garlic for consumption were obtained from the market in Bogor, West Java.

Shallot and garlic bulbs were planted in Plant Virology Laboratory, Faculty of Agriculture, Bogor Agricultural University following growing on test technique. As many as 120 bulb samples from each cultivar were randomly selected, the samples were then divided into 4 parts for each cultivar for the purpose of virus detection as described below. All bulbs were grown on 24 x 32 cm styrofoam plate which was then placed on tray filled with water.

2.2. Detection of viruses using enzyme-linked immunosorbent assay (ELISA) method
Virus detection were conducted for four growing stages of plant i.e. adventitious shoot, shoot, young leaf on 7 days after planting (DAP) and 14 DAP (figure 1). ELISA method was proceeded for detection of GCLV and SLV, i.e. double antibody sandwich-ELISA (DAS-ELISA) and triple antibody sandwich-ELISA (TAS-ELISA), respectively following Dijkstra dan de Jager [10] and Deutsche sammlung von mikroorganismen und zellkulturen (DSMZ) kit protocol.

2.3. Experimental design and data analysis
The experiment was conducted using completely randomized design consisted of 2 factors, i.e. cultivar and growth stage. The cultivars involved in this experiment were shallot cv. Bima Curut, garlic cv. Sembalun, and shallot and garlic for consumption. Four growth stages used in this experiment, i.e. adventitious shoot, shoot, leaf on 7 DAP, and 14 DAP (figure 1). Observation for each stage was repeated 3 times with 10 bulbs for shallot and 10 cloves for garlic.

Disease incidence and virus titer were observed based on ELISA absorbance value ($A_{405nm}$). Means of virus titer were obtained from means of ELISA absorbance value. Percentage of disease incidence were obtained based on formula below:

$$\text{Disease incidence} = \frac{\text{Number of infected samples}}{\text{Number of total samples}} \times 100\%$$
Data analysis was conducted using Microsoft Excel 2010 and software SAS 9.1 followed by Duncan test with 5% significance level.

![Figure 1](image.png)

**Figure 1.** Growth stage of shallot (a-d) and garlic (e-h): (a and e) adventitious bulb, (b and f) shoot, (c and g) young leaf on 7 (DAP) and (d and h) 14 DAP.

3. Results

3.1. Symptoms of virus infection
In general symptoms were developed on 10 DAP but it got more obvious at 14 DAP. The most common symptoms found on shallot included (a) yellow stripe, (b) yellow mosaic, (c) light green mosaic, (d) dark green stripe, (e) wrinkle, and (f) light green stripe (figure 2). Meanwhile the symptoms on garlic involved (a) yellow mosaic, (b) curling on the top leaf, (c) wrinkle, (d) twisting leaf, and (e) yellow stripe (figure 3).

3.2. Incidence of GCLV and SLV infection
Based on ELISA, incidence of GCLV and SLV infection on shallot and garlic showed similar pattern, i.e. high on adventitious shoot, low on leaf shoot, then started to increase again on 7 DAP and 14 DAP leaves (table 1 and 2). Incidence of GCLV infection on shallot did not significantly different on each stage, while incidence of SLV infection were significantly different on leaf shoot and 7 DAP leaves. Incidence of SLV on shallot for consumption was higher than cv. Bima Curut (table 1). Incidence of GCLV and SLV infection on garlic for consumption were higher than cv. Sembalun on adventitious shoot and 14 DAP leaves (table 2).
3.3. Titer of GCLV and SLV
In general, variation in GCLV and SLV titer were observed on each plant stadia. Similar as on disease incidence, titer of GCLV and SLV were high on adventitious shoot, then decreased on shoot leaves, increased again on 7 DAP leaves, and continue increased on 14 DAP leaves (table 3 and 4).

![Symptoms of virus infection on shallot leaves: (a) yellow stripe, (b) yellow mosaic, (c) light green mosaic, (d) dark green stripe, (e) wrinkle, and (f) light green stripe.](image)

**Figure 2.** Symptoms of virus infection on shallot leaves: (a) yellow stripe, (b) yellow mosaic, (c) light green mosaic, (d) dark green stripe, (e) wrinkle, and (f) light green stripe.

**Table 1.** Incidence of GCLV and SLV infection on several growth stadia of shallot.

| Cultivar       | Stadia               | GCLV (%) | SLV (%) |
|----------------|----------------------|----------|---------|
|                | Adventitious shoot   |          |         |
| Cv. Bima Curut | 30.00<sup>a</sup><sup>b</sup> | 0.00<sup>a</sup> | 3.33<sup>a</sup> | 50.00<sup>a</sup> |
| Consumption    | 26.67<sup>a</sup>    | 3.33<sup>a</sup> | 16.67<sup>a</sup> | 63.33<sup>a</sup> |
|                | Shoot                |          |         |
| Cv. Bima Curut | 40.00<sup>a</sup>    | 3.30<sup>b</sup> | 6.67<sup>b</sup> | 70.00<sup>a</sup> |
| Consumption    | 50.00<sup>a</sup>    | 20.00<sup>a</sup> | 36.67<sup>a</sup> | 76.67<sup>a</sup> |
|                | 7 DAP leaves<sup>a</sup> |         |         |
|                | 14 DAP leaves        |         |         |

<sup>a</sup> DAP, days after planting.
<sup>b</sup> Means value followed by the same letter are not significant different at 5% level using Duncan test

Titer value of GCLV and SLV were significantly different on each growth stadia between shallot and garlic from grower and for consumption, except the titer value of GCLV on garlic in leaf shoot stadia. Titer value of GCLV and SLV on shallot for consumption were higher than cv. Bima Curut, except on adventitious shoot (table 3). Similar with titer value of GCLV and SLV on shallot, titer value of GLCV and SLV on garlic for consumption were higher than cv. Bima Curut, except on adventitious shoot and 7 DAP leaves, respectively (table 4).

4. Discussion
Symptoms variation was observed on shallot and garlic, although the most dominant symptoms found were yellow mosaic, green mosaic, and yellow stripe. Similar symptoms were also reported by...
Kadwati and Hidayat [6] on shallot obtained from West Java (Bandung, Cirebon) and Central Java (Bantul, Brebes). Similarly, Fitrasari [11] reported the most dominant symptoms on garlic were light green stripe and curve of the leaf.

**Table 2. Incidence of GCLV and SLV infection on several growth stadia of garlic**

| Cultivar     | Stadia       | GCLV (%) | SLV (%) |
|--------------|--------------|----------|---------|
|              | Adventitious shoot | Shoot | 7 DAP leaves | 14 DAP leaves |
| Cv. Sembalun | 96.67<sup>a</sup> | 16.67<sup>a</sup> | 30.00<sup>a</sup> | 83.33<sup>b</sup> |
| Consumption  | 100.00<sup>a</sup> | 16.67<sup>a</sup> | 30.00<sup>a</sup> | 100.00<sup>a</sup> |
| Cv. Sembalun | 76.67<sup>b</sup> | 0.00<sup>a</sup> | 16.67<sup>a</sup> | 73.33<sup>a</sup> |
| Consumption  | 100.00<sup>a</sup> | 0.00<sup>a</sup> | 16.67<sup>a</sup> | 86.67<sup>a</sup> |

<sup>a</sup> DAP, days after planting.

<sup>b</sup> Means value followed by the same letter are not significant different at 5% level using Duncan test.

**Figure 3. Symptoms of virus infection on garlic leaves:** (a) yellow mosaic, (b) curling on the top leaf, (c) wrinkle, (d) twisting leaf, and (e) yellow stripe.

The result of this research showed variation on incidence and titer of GCLV and SLV according to each growth stage. This condition was also reported by Conci [12] whom found that titer of *Leek yellow stripe virus* (LYSV) on garlic was varied based on cultivar, growth stadia, and growing sites. Titer of SYSV on garlic bulb cultivar Blanco, Mendoza, Norteno INTA, Colorado El Nevado, and Colorado Payén after dormancy period were higher than those on dormant bulb. Wulandari [13] found more positive reaction to GCLV, SLV, and OYDV antibody from leaves samples at 30 DAP compared to bulb samples. Variation of virus titer during different growth stages also occurred in
some plant viruses, such as Prunus necrotic ringspot virus (PNRSV) on cherry and peach plants [14] [15] and Cowpea chlorotic mottle virus (CMV) on soybean [16].

Based on ELISA detection method, the best result for detection of GCLV and SLV on shallot and garlic is using samples from adventitious shoot and 14 DAP leaves. The highest virus titer was found in these growth stages because virus infection on shallot plant will be accumulated from one generation to the next generation through the bulb [6]. Furthermore virus on bulb will multiply along with plant growth, therefore virus titer continue to increase until 14 DAP.

**Table 3. Titer of GCLV and SLV on several growth stadia of shallot**

| Cultivar       | Stadia                | GCLV ($A_{405nm}$) | SLV ($A_{405nm}$) |
|----------------|-----------------------|--------------------|-------------------|
|                | Adventitious shoot    | Shoot              | 7 DAP leaves$^a$  | 14 DAP leaves |
| Cv. Bima Curut | 0.22$a^c$             | 0.096$b$           | 0.125$b$          | 0.222$b$    |
| Consumption    | 0.176$b$              | 0.147$a$           | 0.184$a$          | 0.318$a$    |
| Cv. Bima Curut | 0.324$a$              | 0.079$b$           | 0.177$b$          | 0.243$b$    |
| Consumption    | 0.211$b$              | 0.17$a$            | 0.239$a$          | 0.676$a$    |

$^a$ DAP, days after planting.

$^b$ Means value followed by the same letter are not significant different at 5% level using Duncan test.

**Table 4. Titer of GCLV and SLV on several growth stadia of garlic**

| Cultivar       | Stadia                | GCLV ($A_{405nm}$) | SLV ($A_{405nm}$) |
|----------------|-----------------------|--------------------|-------------------|
|                | Adventitious shoot    | Shoot              | 7 DAP leaves$^a$  | 14 DAP leaves |
| Cv. Sembalun   | 0.432$b^c$            | 0.168$a$           | 0.191$b$          | 0.699$b$    |
| Consumption    | 0.693$a$              | 0.164$a$           | 0.25$a$           | 0.757$a$    |
| Cv. Sembalun   | 0.250$b$              | 0.121$b$           | 0.181$a$          | 0.371$b$    |
| Consumption    | 0.443$a$              | 0.139$a$           | 0.156$b$          | 0.401$a$    |

$^a$ DAP, days after planting.

$^b$ Means value followed by the same letter are not significant different at 5% level using Duncan test.

Incidence of GCLV and SLV on shallot and garlic bulb indicated that the bulb has the potential as primary inoculum sources. It was reported by Kadwati and Hidayat [6] that disease incidence caused by SLV and Potyvirus on shallot in Brebes reached 92.9%, and those on garlic in Bandung reached 42.9%. This result indicated high virus infection in the field. The sources of primary inoculum in the field might come from seed bulbs, while secondary infection might occurred through transmission by insect vector in non persistant manner [3].

Incidence of GCLV and SLV infection on shallot and garlic seed bulbs obtained from seed grower (cv. Bima Curut and cv. Sembalun) and consumption bulbs obtained from market were not significantly different. However, virus titer on shallot and garlic for consumption were higher than those on seeds bulb. Those result needs to be confirmed due to differences in post harvest treatment between the bulb for seed and consumption. Subagyo [17] showed that storage condition and duration of shallot in Brebes did not affect the incidence of OYDV, GCLV, and SLV, although it affected plant growth.
5. Conclusion

Virus detection is recommended at the right stages, i.e. adventitious shoot and 14 DAP leaves because at this stages the titer of GCLV and SLV was the highest. High incidence and titer of GCLV and SLV indicated the potential of seed bulbs as the sources of primary inoculum in the field.

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