Growth and yield response of a local pepper (Capsicum annuum L.) accession to begomoviral caused disease in the medium elevation land

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Abstract. The experiment aimed to evaluate resistance of a local pepper accession to begomovirus caused disease which is annually observed in pepper cultivation in Aceh, Indonesia. The evaluation was conducted by assessing the growth and yield response of the pepper plants in the median elevation. The field experimental was conducted in Agricultural Staff Training Center (BLPP) Saree and Horticultural Laboratory of Syiah Kuala University from December 2018 to August 2019. Purpose sampling method was established to for plant selection devided into two clusters. The first cluster of 930 plants was evaluated for typical symptom of begomovirus caused disease from 15 to 135 days post transplanting (dpt). Second clusters plants were selected and divided into two group of early-symptomatic and late-symptomatic plants consisted of 12 plants, respectively for polymerase chain reaction (PCR) based begomovirus diagnosis and yield survey. Typical begomovirus symptom started to be observed at 30 dpt in small number of plants (3%) and reach the highest rate (100%) at 105 dpt. The difference productivity of early-symptomatic plants was 0.26 tons ha⁻¹ higher than late-symptomatic plants. Begomovirus species which prevalently infect the plants both early and late symptomatic plants was pepper yellow leaf curl Indonesia virus (PepYLCIV).

1. Introduction
Pepper (Capsicum spp.) is a member of Solanaceae which is originated from Central and South America [1]. Among all the domesticated species, C. annuum is the most cultivated. In Indonesia, 2.5 M tonnes of pepper comprising C. annuum and C. frutescens were produced in 2019 to fulfill high demand of local market [2]. Pepper cultivation is concentrated in Java and Sumatera as the main producing area, and it occupies more than 155,000 hectares land in the country to become source of income to more than 500,000 local farmers [3]. Cultivation of pepper in Aceh only contributed to 5% of national fresh pepper production despite being one of the most important condiment cash crops because throughout the year, the demand is always increasing [4].
Parallel with the high demand, pepper is vulnerable to multitude biotic-stress caused by pest and disease [5]. One of the most destructive disease in pepper cultivation is the pepper yellow leaf curl disease (PepYLCD) which can cause initial damage from nursery to harvesting period [6]. PepYLCD is mainly associated to viruses belonging to the genus begomovirus of Geminiviridae family [7]. Begomovirus is actively transmitted by highly polyphagous insect-vector Bemisia tabaci in circulative persistent manner [8]. Typical symptoms of begomovirus infection are yellowing and curling on the leaves followed by the failure of flower and fruit formation [9]. Four out of five pepper cultivation fields in Aceh were observed with typical yellow leaf curl symptom more than 81% in 2012 with the typical causal agent of infection including pepper yellow leaf curl Indonesia (PepYLCIV), and ageratum yellow vein virus (AYVV) [10]. Putative recombinant species of pepper yellow leaf curl Aceh virus (PepYLCAV) was observed in 2017 and became prevalent beyond its putative major parent PepYLCIV [11]. In addition, begomovirus caused disease is not only contributed to vegetative stage of growth with 53.5% decrease but also generative stage which represent by the 72.6% productivity decrease [12].

The current disease management of begomovirus rely mostly on the vector population control using chemical based pesticide which is cost intensive and biohazard [13]. Due to insufficient management measures, the use of resistance materials is necessary to overcome the constraint because it enables cost-effective, long-lasting, and sustainable measure of pathogen control [14]. Accordingly, to assess the proper resistance trait of Capsicum, we conduct field-based experiment to investigate the growth and yield response of a locally grown resistance C. annuum accession.

2. Materials and Methods
2.1. Research and plant material
The experiment was conducted at the Agricultural Staff Training Center (BLPP) Saree, Lembah Seulawah, Aceh Besar. Analysis of growth and yield were conducted at the Horticulture Laboratory of Agrotechnology Departement, Faculty of Agriculture, Syiah Kuala University, Darussalam Banda Aceh. The study was started from December 2018 to August 2019.

2.2. Plant cultivation
A locally cultivated pepper (C. annuum) accession Perintis was used as the plant material. Pepper plants were sown by the farmers in plots with measurements of 50 x 1 m and the total of 8 plots. Drainage formed for the distance of each plot was 50 cm. At the beginning of plantation, 20 tons ha$^{-1}$ of organic fertilizer was applied, followed by the application of inorganic fertilizer for every 7 days as much as Urea 7 kg ha$^{-1}$, NPK 17 kg ha$^{-1}$, KCL 15 kg ha$^{-1}$. Irrigation was maintained for every morning, while controlling the pests was conducted with chemical pesticides.

2.3. Samples selection and data collection
Plant selection was conducted establishing Purpose Sampling method by dividing the plants into two clusters. The first cluster of 930 plants was evaluated for typical symptom of begomovirus caused disease every 15 started from 15 to 135 days post transplanting (dpt) days followed by daily field temperature record by using T&D Thermo Data Logger (TR-71wf).

The second cluster consisted of 12 early-symptomatic plants which was observed at 45 dpt and 12 late-symptomatic plants which was observed at 60 dpt. The second cluster was established to evaluate the growth, yield, and to conduct upper young leaf samples collection for PCR begomovirus diagnosis. The growth parameters observed were consists of plant height (cm), stem diameter (cm), number of secondary branches and percentage of symptomatic leaves. Yield parameters observed were consists of weight per fruit (g), fruit diameter (cm) and plant productivity (ton ha$^{-1}$) from a total of 10 harvesting.
2.4. Begomovirus diagnosis
Upper young leaf samples from the second cluster plants were collected analyzed by conducting conventional PCR to diagnose the infection of predominant infecting species in the field PepYLICIV, PepYLCAV, and AYVV according to annually conducted field experiment since 2012 [10][11].

3. Results and discussion
3.1 Percentage of plants possessing begomovirus typical symptoms
The result of symptomatic plant record (Figure 1) assessed according to observed begomovirus typical symptoms (Figure 2) indicated that the plants started to show typical symptom at 30 dpt by 3%, and 32% transition from healthy to symptomatic plants occurred between 45 to 60 dpt. At 105 dpt, pepper plants had been showing 100% symptomatic condition. As the age of the plants increase, the number of plants possessing symptoms is increasing as well due to the spread of the virus in each growth stage of plants is mainly affected by the spread of vector insects [3].

![Figure 1. Percentage of symptomatic pepper plants from 15 to 135 dpt.](image)

![Figure 2. Typical symptom of begomovirus caused disease in pepper plants, a.) vein swelling, b.) curled and yellowing leaves, c.) very heavy symptom with complete curled leaves and dwarf phenotype of plant.](image)

The observation of quite early begomovirus typical symptoms in more than half of the cultivated plants at 75 dpt could be associated to the high transmission exposure of whitefly vector. In our experimental field, the pepper cultivation was conducted in monoculture pattern annually with almost no rotation of commodity. In addition, the surrounding areas were cultivated with same crops from same family or different crops from different family but possible to become alternative host of the whiteflies or begomovirus. Since *B. tabaci* is a polyphagous insect pest that has approximately 600 host plant species [3], we suspected the early
occurrence of begomovirus typical symptoms in pepper due to the high propagation and transmission activity of the vectors.

Symptoms of *Begomovirus* infection are somewhat vary; depending on the virus strain, genotype, temperature, humidity, topography, vector insects activity and the age of the plant at the time it get infected. Begomoviruses which infect the plants with cultivars or varieties that quite resistant against viral infection are usually shows no symptoms or merely mild symptoms such as slight leaf chlorosis [15]. In accordance with [16] Ochoa-Alejo and Ireta-Moreno (1990) statement, genotypes or varieties that shows resistance against viral infection are able to restrict the development of virus inside the cells and tissues so that the virus does not spread to other cells. Notably, since we did not conduct any disease severity assessment, additional explanation of how severe the typical symptom in Perintis besides the incidence is yet to be elucidated. The changes in leaf shape due to viral infection can particularly be seen in (Figure 2).

3.2 *Begomovirus* infection detection

According to the conventional PCR for virus diagnosis conducted by using leaf samples collected from early-symptomatic (45 dpt) and late-symptomatic (60 dpt) plants, *PepYLCIV* was prevalent infecting species for both of group with the infection rate reaching 100% (Table 1).

| Begomovirus Species | early-symptomatic 45 dpt | late-symptomatic 60 dpt |
|---------------------|--------------------------|-------------------------|
| *PepYLCIV*          | 100                      | 100                     |
| *PepYLCAV*          | 0                        | 8                       |
| *AYVV*              | 0                        | 0                       |

*PepYLCIV* spread rapidly in pepper plants presumably due to begomovirus genetic material is transmitted by whitefly through stylet to the plant cell cytoplasm, some of the virus present at the whitefly body would mixed with the cytoplasmic. Afterwards, cytoplasmic fluid will return into the cell because the air pressure outside the cell is rather higher [17]. When the virus particles has successfully penetrated into the host cell, the nucleic acid would be released from the mantle cell. Nucleic acid itself plays a crucial role for the success of the viral infection process. Hence virus supposed to have an intact nucleic acid genome in order to carry out the replication process [18]. Simultaneously the virus particles will enter the trichome basals, then the virus particles shall be carried by the cytoplasmic fluid to another plant cells. This could affect the host metabolisms and lead the yield production in plants to decrease drastically [19].

*PepYLCIV* is one of the most dominant species of *Begomovirus* infecting chilli pepper plants in Indonesia [3]. At least 4 out of 5 chilli pepper plantations in Aceh are infected by yellow curl disease which caused by *PepYLCIV* [10]. However within five years, recombinant species of *PepYLCIV* was detected in the same chilli pepper plantations with 87% similarity in DNA-A and known as *PepYLCAV* [14]. Eventhough we has no definate elucidation whether *PepYLCAV* has emerged at the begining, its presence represents the ability of a virus in mutation in order to adapting to the environment and establishing infections in host plants with a wider and more broad spectrum [20]. Further monitoring towards the pathogenicity and infection of *PepYLCIV* and *PepYLCAV* indeed indispensible to determine the dynamisms of the pathogen development that inflict on yellow curly disease in Aceh.
3.3 Observation of field average temperature

Figure 4 indicated that the lowest average temperature was occurred at the age of 45 dpt (23.58°C). Whereas the highest average temperature was occurred at 60 dpt (29.35°C), the difference in temperature changes between 45 to 60 dpt was 5.77°C. The result demonstrated that a significant change in temperature can increase the relative humidity (RH) in the field, and this will eventually increase symptomatic condition of plants caused by begomovirus due to the escalating propagation rate of insect vector (Figure. 3).

![Figure 3. The average temperature during experiment period.](image)

The fluctuation in temperature at certain plant stages accelerates the reproduction of whitefly as the virus vector. Drastic changes in temperature was occurred at 45 to 60 dpt (Figure 3) consequently the percentage in viral symptomatic rate at that age increased by 32% (Figure. 1). The drastic increase in viral infection is strongly influenced by significant temperature changes [21]. An epidemic disease outbreak, where the pathogen in this case is the virus, must be virulent; whereas the host plant is supposedly susceptible and environmental conditions such as air humidity, soil moisture, acidity (pH) and soil type must be quite appropriate [22].

3.4 Growth and yield of chilli pepper plants infected with begomovirus

Early-symptomatic plants (45 dpt) had slightly smaller number in of plant height, stem diameter, weight per fruit and fruit diameter than with the late infeted plants (60 dpt) (Table 3). Nevertheless, the number of secondary branches, the percentage of leaves infected by the virus and productivity disclosed that the early-symptomatic plants (45 dpt) generate higher yield than the late-symptomatic plants (60 dpt).

In general, the earlier the infection occurs, the earlier the damage to the plant as well as the decrease in yield. However, in Perintis, the damage to plant phenotype has no obvious effect especially on decreasing crop productivity. The productivity of chilli pepper plants in early infected (45 dpt) is 0.26 ton ha⁻¹ higher than the late infected (60 dpt). Both of the early and late symptomatic plants were supposedly to be suppressed due to the infection of begomovirus and should have lower productivity than average (7-8 ton ha⁻¹) [23]. According to our evaluation, regardless of how early the symptom or infection observed, Perintis remains with generating the approximate number of normal productivities which is an attractive desirable as a cultivar.
Table 2. Growth and Yield Parameters of Chilli Pepper Plants Infected with \textit{Begomovirus}.

| Parameters Growths and Yields | Symptomatic observation | Early-symptomatic | Late-symptomatic |
|------------------------------|-------------------------|-------------------|------------------|
| Plant height (cm)            | 72.40                   | 76.70             |
| Stem diameter (cm)           | 1.128                   | 1.717             |
| Number of secondary branches | 6.33                    | 5.33              |
| Percentage of symptomatic leaves (%) | 60.95               | 59.44             |
| Weight per fruit (g)         | 4.13                    | 4.20              |
| Fruit diameter (cm)          | 0.88                    | 0.89              |
| Productivity (ton ha$^{-1}$) | \textbf{7.71}          | \textbf{7.45}     |

As a locally cultivated accession, Perintis can maintain normal productivity even though it is positively being infected by begomovirus in early growth stage according to the PCR-based diagnosis. Some genotypes certainly have proper pathogen defense system which enable them to maintain productivity [24]. Perintis is a valuable \textit{Capsicum} accession conserved with begomovirus resistance gene \textit{pepy-1}, this further clarify our current evaluation of high capability of Perintis expressing defense system against begomovirus and resulted in higher vegetative growth and productivity [25, 26].

4. Conclusions

The highest percentage of viral infections was appeared at the age of 105 dpt by the number of 100%. Significant changes in environmental temperature between the age of 45-60 dpt had consequence in increasing of viral infection up to 32%. The productivity of early infected (45 dpt) and late infected (60 dpt) has a total of 0.26 tons ha$^{-1}$ difference. Apparently, the most common begomovirus species infect chilli pepper plants is PepYLCIV.

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