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Tobacco Mosaic Virus (TMV) Infection in Several Varieties and Ages of Tomato Plants (*Lycopersicon esculentum*, Mill.)

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Abstract

Disease caused by Tobacco Mosaic Virus (TMV) is an important disease in tomato plants, and is transmitted through both natural and artificial wounds. TMV attacking tomato plants can cause qualitatively and quantitatively lower production. The decline in production by TMV attack can reach 60 %. This research was conducted at the greenhouse of the Plant Protection Department, Faculty of Agriculture, University of Sriwijaya, Indralaya, Ogan Ilir, South Sumatra, Indonesia, from January to June 2016. The purpose of this study was to assess TMV attack on different varieties and ages of tomato plants. This study was conducted using a factorial in Factorial Randomized Complete Block Design (FRCBD), with the first factor, namely plant varieties, and the second factor, age stage of plants, repeated 3 times. The results showed that the most susceptible tomato was "Permata", with the fastest incubation period and moderate disease severity. Age 8 weeks, when inoculation took place, was the most vulnerable time, with the highest disease severity. A combination of the tomato variety of Permata and an age stage of 8 weeks at the time of inoculation had the highest disease incidence.

Keywords: Tobacco Mosaic Virus, plant ages, varieties of tomatoes

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop and planted widely throughout the world, including Indonesia. This crop is a horticultural commodity with high economic value and requires serious treatment, especially in terms of increased production and fruit quality. As a tropical country, Indonesia has the potential, and opportunities that are large enough, to take advantage of business opportunities in the field of horticulture, particularly tomato [1].

According to [2], the production of Indonesian tomatoes in 2014 was as much as 915,987 tons, or about 7.69 percent of the national vegetable production, which ranks the fifth. The Indonesian tomato production center is Java, with a total production of 434,202 tons, or about 47.40 percent of the total national production of tomatoes. West Java is the largest tomato producer, with a production of 304,687 tons, or 33.26 percent of the total national production of tomatoes. Tomato production in 2014 decreased by 7.74 % compared to production in 2013 [2]. In addition to the reduction of land, there are obstacles in the field, such as pests and virus diseases, among others. Low tomato yield is due to a number of factors, i.e., lack of improved well-performing varieties, poor fruit setting by heavy rain, and high temperatures which limit pollination, more specifically, fecundation plus pollen viability, pests, and diseases [3]. Generally, a number of viruses singly infecting the host is known to have the potential to inflict severe losses [4].

In Indonesia, viruses that are attacking tomato plants are the Tobacco Mosaic Virus (TMV), Potato Virus Y (PVY), and Cucumber Mosaic Virus (CMV). These 3 viruses together can reduce production by up to 50 %, depending on the age and variety of the plant when infected [5,6]. TMV can result in poor fruit quality and reduce the value of the crop on the commercial fresh market and may reduce the production by 20 - 60 % [7,8]. This virus, which is seed-borne, is commonly found in tomato plants, and can be transmitted both mechanically or by seed [9,10]. Damiri [11] stated that, in the field, the virus can persist in soil or tobacco debris or in an alternate host. Many factors influence the development of TMV in tomato plants. This paper reports a study on the effect of TMV infection at various ages of 3 tomato varieties.

Materials and methods

This research was carried out in a greenhouse at the Plant Pests and Disease Department, Faculty of Agriculture, University of Sriwijaya, Indonesia, between January and June 2016. The research was arranged in a Factorial Randomized Complete Block Design (FRCBD), with plant varieties as the first factor, and plant age stages as the second factor. The first factor consisted of 3 local varieties, Chung, Tysera, and Permata, whereas the second factor consisted of 4 levels, 2 weeks after transplanting (WAT), 4 WAT, 6 WAT, and 8 WAT. Each treatment was replicated 3 times, and each replicate consisted of 2 polybags, with each polybag containing one tomato plant.

Preparation of host plant and inoculums

Each tomato variety seedling was grown until 2 weeks old and then transplanted into polybags containing 10 kg of sterile mix of topsoil and organic soil (2:1). Inoculum was prepared by fresh freezing TMV symptomatic tissue, which was derived from the Bandung Research Centre for Vegetable. The inoculum was put in liquid nitrogen, then homogenized in cold phosphate buffer, pH 7.2 (1:10 weight by volume). Inoculation was conducted mechanically by rubbing a single young leaf, which was earlier dusted with carborundum 600 mesh, with inoculums of the respective virus. Plants were maintained, including by fertilizing, watering, and weeding.

The parameters observed in this research were the incubation period, disease severity, and first production (number of fruits and the weight of total fruits yield) for each plant. The intensity of virus attacks was calculated using the formula from [12], as follows;

$$I = \frac{\sum n.v}{N.V} \times 100 \% \quad (1)$$

where: I = intensity attack symptoms

n = number of plants included in a particular symptom scale

v = value of certain symptoms score

N = number of plants observed

V = value of the highest severity score

Symptom severity scores were classified as follows:

0 = plants not showing symptoms

2 = plants showing mosaic and yellow groove visible (> 25 - 50 %)

3 = plants showing mosaic and changes in growth (> 50 - 75 %)

4 = plants showing symptoms of severe mosaic, deformed growth, and dwarf plants (> 75 - 100 %)

The data were analyzed using the analysis of variance (ANOVA), and comparison among means with Duncan's Multiple Range Test (DMRT) [13].

Results and discussion

The results analysis of the overall effect in this research showed that varieties and plant age stages significantly affected the incubation period, disease severity, and plant production. The interaction of the tomato varieties and the age stages significantly affected the disease severity; however, there was no interaction effect between varieties and age stages on incubation period and production (number and weight of fruit yield) (**Table 1**).

Table 1 Summary of effect of Tobacco Mosaic Virus infection on incubation period, disease severity, and tomato production

No	Parameters observed	Treatment		
		Varieties	Age stages	Varieties*Age stages
1	Incubation period	**	**	ns
2	Disease severity	**	**	**
3	Production (fruits number and weight)	**	**	ns

Note: ** = significantly different at $p < 0.01$ and ≤ 0.05 , respectively;
 ns = not significantly different

Result of statistical testing showed that tomato varieties and age stages significantly affected incubation period (**Tables 2 and 3**).

Table 2 Effect of tomato varieties on incubation period of Tobacco Mosaic Virus

Tomato varieties	Incubation period (days after inoculation)
Tysera	9.54 a
Permata	10.25 ab
Chung	11.04 b

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)

Table 2 showed that the incubation period on Tysera was not significantly different from Permata, but significantly different from that of the Chung variety ($P \leq 0.05$). The incubation of Permata was not significantly different from that of the Chung variety. The disease symptoms showed by 3 tomato varieties ranged from necrosis to mild mosaic, mosaic symptoms on leaves visible, initially green leaves turned into yellowish, leaves undergoing chlorosis, leaves rather wrinkled, and malformations (see **Figure 1**).



Figure 1 Symptom of *Tomato Mosaic Virus* on tomato leaves

Table 3 shows that the incubation period on a tomato plant when inoculated at the ages of 8 WAT, 6 WAT, and 4 WAT was not significantly different from one to another, but significantly different from that of the age of 2 WAT. Age of tomato plants when inoculated at the age of 8 weeks showed the fastest incubation period, with a time of 9.33 days, while the longest incubation period of time occurred at 2 WAT, being 12 days.

Table 3 Effect of tomato age stages on incubation period of Tobacco Mosaic Virus

Age stages	Incubation period (days after inoculation)
8 weeks after transplanting (8 WAT)	9.33 a
6 weeks after transplanting (6 WAT)	9.56 a
4 weeks after transplanting (4 WAT)	10.22 a
2 weeks after transplanting (2 WAT)	12.00 b

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)

Factors that affect the fast or slow incubation period of the plants include the factor of age of the plant. Beside the age of plant, incubation time was also affected by host factor, virus concentration, environmental factor, virus character, virus development speed in tissue, and plant susceptibility toward infection [14]. According to Kusumawati *et al.* [15], the younger the age of inoculation, the faster the incubation period. However, in this study, what happened was the opposite, where the fastest was on the tomato age of 8 WAT. It is presumed that susceptibility of tomato plants to TMV infection was at older than 8 WAT, because at the time of inoculation TMV, the tomato plants were already flowering, so requiring a lot of energy, while at the same time having infection by TMV being present. Decrease in plant susceptibility at the time of flowering and fruiting was possibly due to the fact that at the time more metabolism processes were being used on the process of flowering and fruiting. At 8 WAT, the tomato plant requires more energy for the flowering and fruiting for production processes, resulting in a lowering of the plant susceptibility. According to Scott *et al.* [16], if the development of early flowering plants is retarded by suboptimal growth conditions, inoculated plants appear more susceptible to the virus, and systemic infections become more widespread. In addition, in a certain plant, there is an anti-virus factor (AVF) [17].

Anti-virus factor (AVF) is one of the factors that affect resistance and vulnerability. AVF that was present during the vegetative phase can suppress the replication of viruses in plants. In contrast, during the phases of flowering and fruiting formation in general, AVF is at minimum, as growth regulators such as IAA (Indole acetic acid) and BAP (benzylaminopurine) get reduced. The decrease in plant ability to produce AVF causes inability in the plants to prohibit virus multiplication [17,18].

Further tests of the effect of TMV infection on tomato varieties, age stages, and the interaction between varieties and age stages were significantly different (Tables 4 - 6). Table 4 showed the disease severity development from the first week to the fifth week. It was clear that the highest disease severity at the fifth week was found in the Permata variety, which was also significantly different ($P \leq 0.05$) from that of the Tysera and Chung varieties, respectively. Between the Tysera and Chung varieties, there was no difference in disease severity from one to another.

Table 4 Effect of TMV infection on development of disease severity on tomato varieties

Variety	Disease Severity at week (%)				
	1	2	3	4	5
Tysera	3.16a	3.90a	4.32a	5.38a	7.46a
Chung	8.74ab	9.42a	11.32a	13.60ab	17.04a
Permata	15.05b	17.34b	19.95b	21.82b	28.73b

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)

The severity of TMV on the tomato variety of "Permata" indicated that the severity of the attacks is higher compared to Tysera and Chung, allegedly because each tomato variety has different levels of resistance against TMV infection. It can be said that Permata is more susceptible to TMV infection. This is reinforced by the statement [19] that different varieties resulted in differences in regulatory genes that affect the level of resistance of the plant susceptibility to viral infections. On the fifth week of observation, disease severity in tomato varieties that were inoculated could be seen; plants inoculated at the age of 8, 6, and 4 weeks after transplanting were not significantly different from each other, but significantly different to disease severity in plants inoculated age of 2 weeks (Table 5).

Table 5 Influence of TMV infection on various age stages on development of disease severity tomatoes

Age stages (WAT)	Diseases severity at week (%)				
	1	2	3	4	5
2	2.71a	3.93a	5.48a	6.51a	7.62a
4	5.10a	6.25a	8.02a	9.61ab	13.41ab
6	10.92ab	12.42ab	14.50ab	17.99bc	23.23b
8	17.21b	18.29b	19.45b	20.35c	26.72b

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)

In **Table 6**, the influence of the interaction between tomato variety and age stages on last observation showed that the highest disease severity found at the combined treatment of Permata and age stage 8 WAT (M3D8) was 42.94 %, while the lowest disease severity occurred in the combined treatment between Chung at age of 2 WAT (M1D2), at 1.99 %. This fact showed that Permata variety inoculated at the age of 8 WAT was very susceptible to TMV infection. Siadi *et al.* [20] suggested that in plants showing symptoms of viral infection, a disruption in the system of metabolism affects the rate of photosynthesis by decreasing the amount of chlorophyll, increasing respiration, increasing the activity of the enzyme, or lowering the amount of plant growth regulator, which may affect the functional systems of plant cells directly or indirectly. Some symptoms of viral infection are the results of alteration to plant growth and development [21].

Table 6 Effect of interaction between variety and plant age at inoculation on disease severity

Interaction	Disease severity at week (%)				
	1	2	3	4	5
M1*D2	0.95a	1.25a	1.44a	1.66a	1.99a
M2*D2	2.30ab	2.59a	3.17a	3.84a	3.98a
M2*D4	2.49ab	3.58a	4.06a	5.31a	7.16a
M2*D8	2.64ab	3.63a	3.89a	5.12a	6.66a
M3*D2	4.89abc	7.69ab	11.82abc	14.04abc	16.91abc
M2*D6	5.20abc	5.92ab	6.17ab	7.23ab	12.07ab
M1*D4	5.28abc	6.19ab	9.32abc	11.74ab	14.38abc
M3*D4	7.52abc	8.99ab	10.69abc	11.78ab	18.67abc
M1*D6	10.49abc	10.78ab	14.03abc	16.86abcd	21.22abcd
M3*D6	17.08bcd	20.57bc	23.33cd	29.70cd	36.41cd
M1*D8	18.25cd	19.50bc	20.50bcd	24.15bcd	30.58bcd
M3*D8	30.74d	32.12c	33.95d	31.78d	42.94d

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)
M1: Chung, M2: Tysera, M3: Permata and D2 = 2WAT, D4 = 4WAT, D6 = 6WAT, D8 = 8WAT

Curver and Padmanabhan [22] stated that there are connections between the interaction of specific virus factor with cell components and alternation in hormone synthesis and signaling. Interaction between the helicase domain of TMV replicase and several numbers of the auxin/Indole acetic acid (AUX/IAA) protein family have been reported [23]. The subcellular localization of these AUX/IAA proteins is altered, and their levels of accumulation lowered in the presence of TMV replicase. On other hand, their partial down regulation through virus-induced gene silencing gives rise to symptoms similar to those of TMV infection. Interaction of AUX/IAA proteins significantly lowers virus accumulation in mature plant leaves [24].

Total and weight of fruits

Further tests (**Table 7**) showed that, in the 3 varieties of tomato tested, the varieties of Permata and Tysera were not significantly different, but significantly different from the variety Chung. Total fruit weights of each variety were significantly different from each other.

Table 7 The effect of TMV inoculation on first production of tomato varieties

Tomato varieties	Total fruits/plant (number)	Total weight of fruit/plant (g)
Permata	1.80a	44.22c
Tysera	2.25a	78.25b
Chung	3.25b	17.8a

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)

A further test of the influence of the age of the plant at the time of inoculation to TMV on the number and total fruits/plants showed that plants inoculated at the age of 2 weeks had the highest total number and weight of the fruit, averaging 4.00 of fruit/plant and 69.40 grams, respectively, significantly different to all the other treatments (**Table 8**).

Table 8 Effect of tomato age stages on TMV inoculation on the production

Age when inoculated	Total of fruits/plant	Total weight of fruits/plant (g)
2 WAT	4.00b	69.40b
4 WAT	2.22a	45.50a
6 WAT	2.17a	38.80a
8 WAT	2.27a	47.90a

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$, DMRT)

Total weight of Chung was lower than the Tysera and Permata; this is because the morphology of fruits of the variety Chung is smaller compared to Tysera and Permata. Viral infection can affect physiological functions of plants. TMV can make alterations in carbohydrate distribution, so that the physiology of plant is disturbed [25]. Kusumawati *et al.* [15] stated that decrease in the weight of the fruit due to the presence of infection TMV makes the magnitude of the weight loss of fruit on each distinct variety possible because each has a level of resilience that differs in receiving the response to viral infection, and with different responses, the course will affect the growth and crop production. According to Taufik *et al.* [26], the inhibition of plant growth response is generally caused by viral replication contained in the plant. Viral replication occurs in both the inoculated part and in the part of plants that are not inoculated. It can even enter the transportation system of plants, so that the virus can spread systemically throughout the plant, resulting in the plants having stunted growth. Virus infection usually causes symptom resulting both in morphological and physiological alteration of the infected host plants, which always incurs inferior performance, such as decreased host biomass and crop yield loss.

Conclusions

From this study, it can be concluded that the most susceptible tomato variety is "Permata", with the fastest incubation period. Age 8 weeks when inoculation took place was the most susceptible, with the highest disease severity. A combination of tomato variety Permata and the age stage of 8 weeks at the time of inoculation has the highest disease severity. The Chung variety has the highest amount of fruit, but the least total weight fruit/plant.

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