Aphids Identification and Ability Test in Transmitting Zucchini Yellow Mosaic Virus (ZYMV)

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Abstract. Zucchini (Cucurbita pepo L.) is a type of highland vegetable that has high economic value for farmers, but currently, farmers suffer losses due to the Zucchini Yellow Mosaic Virus (ZYMV) with a disease percentage of up to 89.80%. This study aims to identify the type of aphids as a ZYMV vector and determine its transmission potential. This research is an experimental study carried out in the laboratory and the field. Aphids were identified using the Blackman and Eastop identification keys. Serological tests to detect ZYMV are carried out using Enzyme-Linked Immunosorbent Assay (ELISA) with specific antiserum ZYMV. The results showed that there were three types of aphids as ZYMV vectors in the field, namely Aphis gossypii, Myzus persicae, and Aphis craccivora. Potential transmission of ZYMV from three different aphids, namely A. gossypii has the highest transmission ability of up to 70%, then M. persicae shows the transmission ability of 40% and A. craccivora shows the lowest transmission ability of 20%.

1 Introduction

Zucchini is a vegetable of the family Cucurbitaceae by the name of the species Cucurbita pepo L. Zucchini plants were relatively new known in Indonesia in the mid-20th century and began to enter the centers of highland agriculture in Indonesia. Zucchini plants can grow and produce well in areas that have a temperature of 180 C to 240C, with air humidity between 60% to 90% at altitudes from 600 m to 1.200 m above sea level (Risa, 2014).

The zucchini plants have been intensively cultivated since 2005 in the Baturiti District area of Tabanan Regency, Bali Province, Indonesia. The results of observations at several Zucchini plantations were found to be mosaic disease events with a percentage of disease incidence up to 89.80% and mosaic disease events on zucchini plantations in Bali induced by Zucchini Yellow Mosaic Virus (ZYMV) (Pandawani, 2017).

Symptoms of disease that appear on zucchini plants due to ZYMV induction varies from the occurrence of chlorosis in the shoots, thickening of the leaf bone (vein banding), leaf blister lamina on the green part (blistering), bleaching of the bone (vein clearing), attacks on young leaves cause lamina growth is inhibited or even not formed at all (shoestring) and plants also show symptoms of malformation and distortion of the leaves

and fruit. The occurrence of mosaic diseases that appear in zucchini plantations results in a decrease in crop productivity where the quantity and quality of the fruit produced are very low, incompatible with market demand, and even to the point of crop failure.

The existence of a virus in a particular location is the result of a complex interaction of various physical and biological factors. Physical factors include the environment regarding rainfall, wind temperature, soil, and weather, while biological factors include the nature of viruses, host plants, distribution and cultivation activities, epidemics of plant diseases can occur due to the presence of susceptible hosts, virulent pathogens, insect vector carriers diseases and the environment that support the development of disease in a population in a certain time (Agrios, 2005).

ZYMV is generally transmitted in two ways namely horizontally through aphids vectors, and vertically through transmission from the first generation of seeds infected with ZYMV to the next generation (Simmons et al., 2011; Tobias et al., 2003). The most common virus transmission in the field is transmission through vector insects. Insects that can be virus vectors are generally vectors that have mouth pricking and sucking tools such as aphids, leafhoppers, whitefly, ladybugs, and trip (Nurhayati, 2012). 10 species of aphids can be virus vectors, but A. gossypii and A. craccivora are the most important virus vectors in Hawaii. A. gossypii and M. persicae transmitted the Papaya ringspot virus and Zucchini yellow mosaic virus non-persistently to zucchini (Pinto et al., 2008), and melons (Martin et al., 2003). According to Coutts (2006), ZYMV is transmitted non-persistently by several species of aphids such as A. gossypii and M. persicae.

Aphids which act as ZYMV vectors on Zucchini in Indonesia are not yet known with certainty, as well as ZYMV attacks are still relatively new in Indonesia. Therefore this study aims to identify the type of aphids that play a role in transmitting ZYMV and determine the ability of these vectors to transmit ZYMV to zucchini plants.

2 Methods

This research is experimental in the laboratory and the field. Field research was carried out at the zucchini planting center in Baturiti district, Tabanan regency, Bali province. Laboratory research was carried out at the Denpasar Class I Agricultural Quarantine Laboratory and the Insect Systematic Laboratory of the Plant Protection Department, Faculty of Agriculture, Bogor Agricultural University.

2.1 Propagation of ZYMV inoculum sources

Plant leaves used as samples are leaves that show symptoms of mosaic, necrosis, bleaching of leaf bones, and malformations. The leaves are stored in a freezer at -80 ° C. Serological tests to detect ZYMV are carried out using Enzyme-Linked Immunosorbent Assay (ELISA) with specific antiserum ZYMV, CMV, and PRSV. The source of ZYMV inoculum is propagated through mechanical transmission by applying ZYMV infected leaf juice to healthy zucchini leaves that have been dusted with 600 mesh carborundum (Bos, 1990). Plants that have been inoculated are stored in a greenhouse until symptoms appear.

2.2 Collection and identification of aphids

All types of aphids in Zucchini infected with ZYMV have collected especially the imago stage, maintained in insect cages. Then the aphids were identified using the identification key of Blackman and Eastop (2000), which is based on the morphology of aphids that are not yet winged, especially in the caput, sifunkuli, and cauda.

2.3 Test the potential of aphids in ZYMV transmission

Previously collected aphids were kept on taro plants to free them from ZYMV so that the resulting nymphs were free of ZYMV. This procedure is following the procedure carried out by Sularno, (2009). Newborn aphids (nymphs) produced from virus-free imago are then transferred to healthy zucchini plants in insect cages and allowed to multiply to obtain virus-free nymphs. These aphids are then used to test the ability to transmit ZYMV. Aphids Nymphs are always virus-free (non-viruliferous) (Djikstra & de Jager, 1998).
The nymph aphids were fasted for one hour, then transferred to zucchini plants infected with ZYMV with an acquisition period of two hours. Furthermore, the inoculation feeding period was carried out for 24 hours by removing 10 infective aphids on each healthy zucchini plant. Ten healthy zucchini plants that are one month old are needed to determine the potential transmission of ZYMV from each aphid. The test plants were then stored in an insect cage, and aphids were killed after 24 hours of inoculation feeding. Observations were made every day for up to two months after transmission to determine the incubation period, symptoms, and percentage of disease events. Detection of the success of ZYMV transmission was carried out using an ELISA serology test.

2.4 Serology test by ELISA

ELISA tests were carried out following the protocols from DSMZ (Deutsche Sammlung von. Mikroorganismen und Zellkulturen GmbH) - Germany, using special CMV antiserum (Agdia Inc., USA) and Potyvirus (Agdia Inc., USA). Virus accumulation is read quantitatively using ELISA Reader 550 (Bio-Rad, USA) at a wavelength of 405 nm. ELISA results are positive if the absorbance value of the sample is one and a half or two times greater than the value of negative control absorbance (Matthews, 1993).

3 Results and Discussion

3.1 ZYMV symptoms in zucchini plants

ZYMV symptoms in zucchini found in the field are mosaics, namely dark green and light green on the leaves, thickening of the leaf bones, leaf blister lamina especially the green part (*blistering*), leaf bone color paler (*vein clearing*), leaf lamina is not formed well so that the shape of the leaf is like a shoestring, and the presence of symptoms of malformation and distortion of the leaves and fruit, especially on older plants (Figure 1). Symptoms vary when plants respond to a viral infection, which depends on (1) the type or variety of plants, the age or stage of plant growth (2) the level of viral virulence and the presence of other infections (3) vector insect species and (4) the environment in which plants grow such as humidity, temperature, and rainfall (Agrios, 2005).

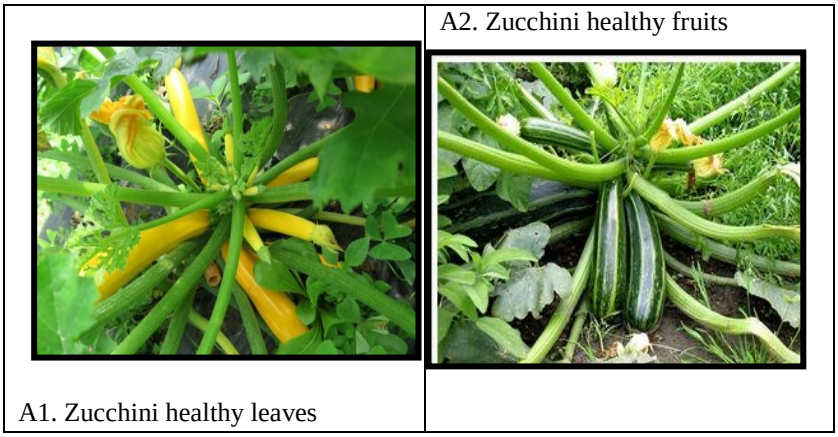

Fig. 1A. Healthy leaves and fruit

Fig. 1B. Malformation and distortion of ZYMV infected on zucchini leaves and fruit

3.2 ZYMV vector identification in the field

The results of identification of aphids colonies in the field using Blackman & Eastop (2000) guidelines, found that the aphids colonies consisted of three aphids species namely *A. craccivora*, *M. Persicae*, and *A. gossypii*. All three types of aphids play a role in transmitting ZYMV. A picture of the aphids colony is presented in Figure 2.

Fig. 2. Aphids colony in zucchini plantations

Three types of aphids that have been identified have characteristics according to the Hemiptera Order and all three have the same characteristics as the genus Aphis. The first aphid that has a blackish-green color (Figure 3 A) is species *A. gossypii*, the second aphid is green (Figure 3 B) is the species *M. persicae* and the third aphids *A. craccivora* has a rather shiny black color (presented in Figure 3).

1. *Aphis gossypii* 2. *Myzus persicae* 3. *Aphis craccivora*

Fig. 3. Three types of aphids that can transmit the ZYMV virus to zucchini plants in Bali, are 1) *A. gossypii*, 2) *M. persicae* and 3) *A. craccivora*

Viral infections cannot occur without other supporting factors such as plant injury or transmitted by vectors. Transmission of the most common virus in the field and the most detrimental is transmission by insects. Insects that can be virus vectors, in general, are those that have a mouth piercing and suction tool. These vectors are aphids, leafhoppers, whitefly, small beetles, ladybugs, and trips (Nurhayati, 2012).

The *A. craccivora* has the main host plant of the Leguminosae family (soybeans, peanuts), turnips, cucumbers, and cabbage. The Cucurbitaceae family (cucumbers, watermelons, melons, and cabbage) are the main hosts of *A. gossypii* which also attacks chili and paprika. Whereas *M. Persicae* attacks more plants from the Solanaceae and

Brassicae families such as potatoes, tobacco, beans, sugar beets, and cabbage (Blackman & Eastop, 2000). Although only *A. gossypii* was recorded as having the main host of the Cucurbitaceae family, all three types of aphids were found to attack zucchini in Bali province.

3.3 Potential of aphids as ZYMV vectors

Three types of aphids namely *A. gossypii*, *M. persicae*, and *A. craccivora* which have been successfully identified and subsequently tested for their ability to transmit ZYMV. ZYMV infection is characterized by mosaic disease symptoms in inoculated test plants and the appearance of symptoms in test plants becomes a parameter of the success of aphids to transmit ZYMV. The results showed that the three types of aphids found attacking zucchini in the field could transmit the ZYMV virus with different abilities. The test results showed that *A. gossypii* had the highest ZYMV transmission effectiveness, which is up to 70%, then *M. persicae* 40%, and *A. craccivora* showed the smallest transmission effectiveness of 20%, as presented in Table 1.

Table 1. Test results ZYMV transmission vectors

Insect Vectors	The incubation period (DAI)	Incidence of disease (%)	*positive reaction ELISA (%)
A. craccivora	14.50	20	20
A. gossypii	12,14	70	70
M. persicae	14, 24	40	40
Control plants	-	0	0

Note: DAI = days after inoculation, * = Antiserum ZYMV

ELISA test results showed that the test plants with a positive reaction to the ZYMV antiserum had variable absorbance values (Table 2). These results illustrate that zucchini plants are infected with ZYMV through transmission by aphids vectors with weak to strong infection rates.

Table 2. ELISA test results to detect the ability of aphids in transmitting ZYMV to zucchini plants

Test plant	Absorbance value ELISA NAE Test (405 nm)		
	A. gossypii	*M. persiceae*	*A. craccivora*
1	0.486 / +	0.225 / -	0.212 / -
2	0,550 / +	0.466 / +	0.216 / -
3	0.426 / +	0.532 / +	0.423 / +
4	0.458 / +	0.212 / -	0.224 / -
5	0.226 / -	0.222 / -	0.452 / +
6	0.511 / +	0.486 / +	0.216 / -
7	0.605 / +	0.202 / -	0.220 / -
8	0.635 / +	0.218 / -	0.218 / -
9	0.238 / -	0.203 / -	0.232 / -
10	0.231 / -	0.476 / +	0.226 / -

NAE average negative control	0.178
NAE average positive control	0.788

Note: ELISA reaction is positive if NAE samples greater than 1.5 x NAE control negative.

According to Matthews (1993), there are several levels of the ability of vectors to transmit viruses, namely: a) Infection is weak (absorbance value of the sample is about twice the control negative), b) moderate infection (absorbance value is half of the control positive) and c) infection strong as indicated by the absorbance value is proportional to the positive control. ELISA absorption value (NAE) of test plants with *A. gossypii* reached 0.635, with *M. persicae* 0.532, while with *A. craccivora* 0.423. These results indicate that *A. gossypii* has the transmission ability of ZYMV with the highest virus concentration results compared to the *M. persicae* and *A. craccivora.* The results of this study are supported by Greber (1988), and Coutts *et al*. (2011) which states that ZYMV transmission through aphids is often transmitted by *A. gossypii* and *M. persicae*. On the other hand, the results of the research by Castle *et al*., (1992) stated that *M. persicae* and *A. gossypii* had a ZYMV transmission efficiency of 41% and 35%, respectively. Furthermore, Glasa *et al.*, (2007) state that ZYMV can be transmitted by *M. persicae* and *A. gossypii* with transmission efficiency reaching 67% and 54%, respectively. Differences in the effectiveness of ZYMV transmission by vectors occur because of differences in interactions between stylet vector receptor proteins and protein coats (CP) and protein-component ZYMV Helper (HC-Pro). ZYMV isolates in the province of Bali have a stronger interaction with *A. gossypii* than with *M. persicae*. This result is reinforced by the statement of Urcuqui-Inchima *et al*., (2001) which states that the transmission of vectors occurs as a result of interactions between the protein receptor stylet aphid, and two viral proteins namely protein coating (CP) and Helper-protein component (HC-Pro), and it is also stated that the role of CP and HC-Pro in the ZYMV genome is closely related to the effectiveness of virus transmission through aphids.

4 Conclusion

1. Types of aphids on zucchini plantations in the province of Bali - Indonesia identified three types and played a role in transmitting ZYMV, namely *Aphis gossypii*, *Myzus persicae*, and *Aphis craccivora*.
2. Potential transmission of ZYMV from three different aphids, namely *A. gossypii* has the highest transmission ability of up to 70%, then *M. persicae* shows the transmission ability of 40% and *A. craccivora* shows the lowest transmission ability of 20%.

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