Research Article

Confirmation on Status of Chaetocnema basalis (Coleoptera: Chrysomellidae) as A Vector of Stewart Wilt Disease

Konfirmasi Status Chaetocnema basalis (Coleoptera: Chrysomellidae) sebagai Vektor Penyakit Layu Stewart

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ABSTRACT

Chaetocnema pulicaria and C. denticulata are recognized as vectors of Stewart wilt disease caused by Pantoea stewartii on maize. These insects have not been reported yet in Indonesia, but Stewart wilt disease has been reported in Java and Sumatera Islands. Genus Chaetocnema which presented in Indonesia is C. basalis. It is not cleared whether C. basalis is a vector for Stewart wilt disease like C. pulicaria and C. denticulata. This reseach was aimed to conduct the confirmation on status whether C. basalis have a role as vector of Stewart wilt disease on maize or not. C. basalis imago were collected from maize growing areas in Yogyakarta, and then starved for 24 h. Treatments were applied by placing imago of C. basalis on infected-P. stewartii plants for 72 h. Five insects were then transferred to each plot of healthy plant (1 plot consisted of 5 plants) for 72 h. For control, imago of C. basalis were put on healthy plants for 72 h and five insects were then transferred to other healthy plant (1 plot consisted of 5 plants) for 72 h. Each treatment was repeated three times. On the fifteenth days after transmission, PCR assays were carried out on leaf samples and isolates of bacteria. All sampled leaves analysis showed that there were no Stewart wilt diseases transmission based on PCR assay and bacterial isolates. This concluded that C. basalis is not a vector for Stewart wilt disease on maize.

Keywords: Chaetocnema basalis, Stewart wilt, vector

INTISARI


Kata kunci: Chaetocnema basalis, layu stewart, vektor
INTRODUCTION

Chaetocnema is a grass flea beetle which belongs to family of Chrysomellidae and distributes almost throughout the world. It is approximately 437 of about 630 species had been identified (Konstantinov et al., 2011). In Indonesia, Kalshoven (1981) reported the presence of genus Chaetocnema populations on paddy, maize and grasses which were assumed as Chaetocnema basalis.

Stewart wilt disease caused by bacterium Pantoea stewartii has generated large problems for maize producing countries such as United States of America (USA), resulting yield loss up to 95%. Currently, this disease has been reported in all over the world such as: Austria, Bolivia, Brazil, Canada, Costa Rica, Guyana, Mexico, Peru, Puerto Rico, USA, China, India, Malaysia, Thailand, Vietnam, and Indonesia (Anonymous, 2016a). In Indonesia, it was considered as new disease on maize and had been reported distributing in Pasaman Barat, West Sumatera (Rahma, 2013), Bogor (Rahma et al., 2014), and Yogyakarta (Anonymous, 2015). Rahma (2013) detected the presence of this disease in maize producing center of West Sumatera with disease incidence ranging 1−15%.

Stewart wilt disease dispersed through seed and vector (Pataky, 2004). Chaetocnema pulicaria and C. denticulata were reported as vectors of this disease (Poos & Elliot, 1936). These two species have not been found yet in Indonesia. Genus Chaetocnema which was found associating with maize in Indonesia is C. basalis. This research was aimed to investigate whether C. basalis that grouped into same genus with C. pulicaria and C. denticulata is able to transmit Stewart wilt disease on maize.

MATERIALS AND METHODS

Research was conducted in a green house, Faculty of Agriculture, Universitas Gadjah Mada, from December 2016 until January 2017. Imago of C. basalis were collected from maize growing areas in Sleman, Yogyakarta (110,4000 BT; 7,7099 LS). Bonanza variety of sweet corn was used as a test plant. The 3-days old bacterial isolate on yeast dextrose calcium carbonate (YDC) medium was then diluted into sterile aquadest to obtain bacterial suspension. Turbidity level of suspension was measured using spectrophotometer at wavelength of 600 nm and OD of 0.3 which was estimated containing bacterial cell about 1.6×10⁹ cell ml⁻¹. The suspension was then injected on 8-days old maize plant, and confirmed to be infected with Stewart wilt through molecular assay (Figure 1), then 24 h-starved C. basalis were put on infected plant for 72 h. Five C. basalis were then transferred to plot of 8-days healthy plant (each plot consisted of 5 covered plants) for 72 h, furthermore considered as Treatment 1 (P1).

The collected and 24 h-starved C. basalis were placed on healthy plant for 72 h. Five insects were then transferred to plot of 8-days plant (each plot consisted of 5 covered plants) for 72 h, here in after used as control (P0), each treatment was repeated three times (Figure 2).

On the fifteenth day after transmission, leaves of each plant were sampled and analysed using two methods, i.e. direct plant extraction and isolation of Nutrient Agar (NA) medium. DNA extraction was performed using method developed by Goodwin et al. (1994).

![Figure 1. Molecular test of artificially inoculated of Pantoea stewartii to plant: 1=plant 1, 2=plant 2, M=1 kb DNA ladder, K+ = positive control](image)

![Figure 2. Flow chart of transmission assay of Chaetocnema basalis against Pantoea stewartii on maize](image)
Leaf samples were washed with running water, surface sterilized with 70% ethanol for 1 min, rinsed 3 times with sterile water, and dried on sterile paper towels. Symptomatic leaf samples were aseptically cut into 10 mm×10 mm pieces and put into NA medium, incubated overnight to allow release of bacteria into the medium. The bacterial suspension was then transferred and purified on YDC, the DNA of 3-days pured cultures were then extracted using CTAB method of Goodwin et al. (1994). Extracted pellet was checked to find out the presence of DNA by mixing the pellet with loading dye in 1:4 ratio, and then put into well of agarose 1% and electrophoreted (50 Volt for 40 min). Afterwards, DNA was amplified according to Coplin et al. (2002), molecular assay was carried out with Polymerase chain reaction (PCR) using primer of HRP3c 5'-GCG GCA TAC CTA ACT CC-3' and HRP1d 5'-CA CTC ATT CCG ACC AC-3', in following mixed composition: 1 µl of DNA pellet, 1 µl of primer HRP3c, 1 µl of primer HRP1d, 12.5 µl of go taq reaction, and 9.5 µl of dd H$_2$O. The protocol were as follows: predenaturation of 94°C for 1 min, denaturation of 94°C for 15 s, annealing of 55°C for 15 s, extension of 72°C for 30 s, and final extension of 72°C for 5 minutes. Steps of denaturation, annealing, and extension were cycled for 25 times.

PCR products were then put into well of 1.5% and electrophoreted (50 Volt for 45 min). Electrophoresis product visualized on UV transilluminator. Percentage of disease incidence was analysed with $t$-test with $\alpha$ level of 5% ($p = 0.05$).

RESULTS AND DISCUSSION

Transmission test showed that C. basalis could not transmit P. stewartii, pathogen of Stewart wilt. Molecular assay as shown on Figure 3, 4, and 5, in which all of samples and analyses methods showed negative results. Figure 3 on test control plants (P0),
indicating negative results of all repetition and tested plant have been extracted and molecular analysed. Figure 4 showed that on treated plants (P1) also revealed negative results in all repetition and tested plant has been extracted and molecular analysed. Figure 5 showed that transmission test on all treatments and isolated plant samples, leaf samples exudating bacterial colony and then purified and extracted. Molecular assay expressed negative results on all sampled isolates.

Disease transmission through vector was a complicated interaction between insect, plant and pathogen (Martini et al., 2015). Feeding manner of insect, immunity reaction of insect and plant, and characteristics of pathogen were some considering factors. *C. basalis* was beetle with mouthparts type of biting and chewing, acquisition of pathogenic bacteria occurred through its mouthpart during feeding process. Transmission happened through saliva containing pathogen which would penetrate via lesion occurring for feeding process, so that the pathogen could spread through xylem vessels and cell cavities (Orlovskis et al., 2015). However, feeding process of *C. basalis* did not generate the infection of Stewart wilt, the occurred lesion was the enter for other pathogen.

The strange objects which entered into insect bodies could be pathogenic or symbiotic (Ammar et al., 2014). They would establish defense systems when foreign objects penetrated into their bodies (Chapman, 2013). The same reaction also occurred on plants when extraneous particles got into their tissues, i.e. utilizing induced resistance systems which were considered as a normal mechanism functioning in restriction of pathogen growth and spread on plant (Agrios, 2005).

*P. stewartii* associated with digestive tracts of insect vectors (foregut, midgut, hindgut, and malpighian tubule). Pathogen could be observed in midgut and hindgut until 12 days after acquisition (Ammar et al., 2014). Pathogen would be brought by blood circulation and dissolved in saliva. Such condition took place when the pathogen was persistent in vector.

*P. stewartii* used two type III of secretion systems (T3SS) to establish colony on host plant and its vector. Colony establishment on host plant was initiated by producing injectisomere or pili to transfer effector protein to host plant. T3SS was important factor for pathogenicity of *P. stewartii* on maize plant. Secretion system of second T3SS was the establishment of *Pantoea secretion island 2* (PSI-2) which was required by pathogen to be persistent in insect bodies. Mutagenesis of *PSI-2* psaN gene was very influential in reducing the persistency of *P. stewartii* in gut of vector and the ability of beetle in transmitting the pathogen on host plant (Correa et al. 2012). The incompetence of *Chaetocnema basalis* in transmitting...
Stewart wilt was much affected by the inability of *P. stewartii* to survive in *C. basalis*.

Generally, interaction of pathogen and vector was specific, in this case, *C. pulicaria* was the specific vector for *P. stewartii*. The presence of other Chaetocnema species in pathosystem of Stewart wilt was not necessarily vector for dispersal of *P. stewartii*. The existence of *C. basalis* on maize was also restricted as herbivores on those plants.

Stewart wilt dispersed through seed and vector, the seed dispersal intensity was less than 0.3%, so that insect vector was considered as main factor in distribution of this disease. *C. Pulicaria* and *C. denticulata* were effectively recognized in dispersing of this disease, and the spread by other vector was assumed less effective (Pataky & Ikin, 2003).

In several areas reporting the incidence of Stewart wilt disease, pathogen could not survive and establish in outside of North America (Pataky & Ikin, 2003). Austria, Greece, Poland, Rumania, dan Russia documented the incidence of this disease, however the pathogen could not survive in those regions. China, Vietnam, Thailand, and Malaysia had also recorded the prevalence of this disease, but the pathogen could not survive as well (Anonymous, 2016b). Pathogen survives in insect bodies and host tissue, those factors should be simultaneously for pathogen to be able to survive and establish in certain areas. Yet found of *C. pulicaria* and *C. denticulata* can be considered in evaluation the status of Stewart wilt disease. Although the disease prevalence has been reported, pathogen can not survive and establish in Indonesia. So that it is required sustainable monitoring of this disease and its vector. Moreover, the improvement of supervision on pathways at entry points should be performed in order to ensure that Indonesia could be free from Stewart wilt disease.

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**LITERATURE CITED**


