IN TR O D U C TIO N

*Ageratum conyzoides* is a member of Compositae Family. *A. conyzoides* as agricultural weed are commonly found in Indonesia and grown well in different types of land such as dry field, paddy field, house yard, and the edge of the highway. The characteristics of *A. conyzoides* are growing upright, cylindrical stems, feathery leaves, and stems, oval and pointed-edge of the leaves, and purplish flowers (3 mm). This weed originates from the tropics area in America (Haselwood & Motter, 1966). In various crops, the presence of *A. conyzoides* potentially causes damage because it can inhibit the growth of major crops through space and nutrition competition, also being an alternative host of pests and diseases i.e. Begomovirus. During the fallow season, *Bemisia tabaci* which is a vector of begomovirus will use *A. conyzoides* as an alternative and reproductive host. *B. tabaci* living in *A. conyzoides* has a short life cycle, a high reproductive rate, and a high fecundity (Subagyo & Hidayat, 2014). Therefore, *A. conyzoides* may potentially be infected by begomovirus and later become inoculum source for the surrounding plants.

Begomovirus is an economically important pathogen in various crop cultivation, especially horticulture and plantation. Begomovirus causes the epidemic of chili leaf curl since 1999 in West Java and 2000 in the Special Region of Yogyakarta (Hidayat et al., 1999; Sulandari et al., 2001), also the epidemic of tobacco leaf curl disease in PTPN X Klaten (Aji et al., 2015). Begomovirus species found in Indonesia are Pepper yellow leaf curl *Indonesian virus* (PepYLCIV), *Tomato leaf curl Kanchanaburi virus* (TYLCKaV), *Tomato leaf curl New Delhi virus* (TYLCNDV), *Ageratum yellow vein virus* (AYVV), and *Tomato leaf curl* *Java virus* (ToLCJaV) (Kenyon et al., 2014; Wilisiani et al., 2014).

Begomovirus is a genus of plant virus that has a single-stranded DNA genome (ssDNA). The Begomovirus genome may possess monopartite genome consisted of DNA-A or bipartite consisted of DNA-B. Each genome sized approximately 2.7–2.8 kbp. DNA–A has six ORFs to release and to form coat proteins; to replicate, to transcript, to transport virus from one cell to other, and to suppress plant defense system (Fontes et al., 2004; Rigden et al., 2004; Hussain et al., 2005; Wang et al., 2013).

Ageratum yellow vein disease (AYVD) is a

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**ABSTRACT**

*Ageratum conyzoides* is a common weed in Indonesia. The weed can decrease plant yield by resource competition and its role as an alternative host of pests and diseases, especially begomoviruses transmitted by whitefly. This research aimed to detect and characterize the begomovirus-betasatellite on *A. conyzoides*. *A. conyzoides* showed severe yellowing symptoms were collected from Magelang, Central Java, Indonesia. Total DNA was extracted and analyzed by PCR for begomovirus and betasatellite. Begomovirus detection was performed by universal primer Krusty-Homer, resulted in a 580bp DNA fragment. Betasatellite detection performed by specific primer β01/β02, resulted in a 1300bp DNA fragment, indicated the presence of a betasatellite associated with the begomovirus. Sequences of begomovirus showed 95% similarity with *Tomato leaf curl Java virus* (ToLCJaV) and betasatellite showed 85% similarity with *Tomato leaf curl betasatellite* (ToLCB). Three main characteristics of betasatellite were TAATATTAC stem-loop structure, Adenine rich region, and ORF BC1 consisted of 118 amino acids.

**Keywords:** *Ageratum conyzoides*, begomovirus, betasatellite, PCR, ToLCJaV

**INTRODUCTION**

*Ageratum conyzoides* is a member of Compositae Family. *A. conyzoides* known as agricultural weed are commonly found in Indonesia and grown well in different types of land such as dry field, paddy field, house yard, and the edge of the highway. The characteristics of *A. conyzoides* are growing upright, cylindrical stems, feathery leaves, and stems, oval and pointed-edge of the leaves, and purplish flowers (3 mm). This weed originates from the tropics area in America (Haselwood & Motter, 1966). In various crops, the presence of *A. conyzoides* potentially causes damage because it can inhibit the growth of major crops through space and nutrition competition, also being an alternative host of pests and diseases i.e. Begomovirus. During the fallow season, *Bemisia tabaci* which is a vector of begomovirus will use *A. conyzoides* as an alternative and reproductive host. *B. tabaci* living in *A. conyzoides* has a short life cycle, a high reproductive rate, and a high fecundity (Subagyo & Hidayat, 2014). Therefore, *A. conyzoides* may potentially be infected by begomovirus and later become inoculum source for the surrounding plants.

Begomovirus is an economically important pathogen in various crop cultivation, especially...
disease caused by begomovirus infection on \textit{A. conyzoides}. The incidence of AYVD can cause the epidemic of the yellow vein disease on Solanaceae, Fabaceae, and Cucurbitaceae cultivation due its potential as initial inoculum of begomovirus infection. AYVD may caused by several viruses including AYVV and ToLCJaV (Saunders \textit{et al.}, 2000; Kon \textit{et al.}, 2006). AYVV and ToLCJaV are begomoviruses that only have a single genome DNA–A (monopartite) and belong to the Old World Begomovirus group. Both viruses are commonly found in the Asian Continent and known infect commercial plants (Kenyon \textit{et al.}, 2014; Kon \textit{et al.}, 2006). Single infection of AYVV without satellite DNA may just induces mild symptom or even asymptomatic, while the common symptoms of AYVV including vein yellowing, mosaic leaves, and curling leaves caused by the association of AYVV associated with betasatellite (Saunders \textit{et al.}, 2004; Briddon & Stanley, 2006; Kon \textit{et al.}, 2006). Meanwhile the association between AYVV and alphasatellite could attenuates symptom severity and reduces begomoviral-betasatellite accumulation (Idris \textit{et al.}, 2011).

Betasatellite, alphasatellite, and deltasatellite are satellite DNA associated with several species of begomovirus. Those satellites have a circular DNA genome and a conservative region with TAATAT TAC stem-loop structure (Zhou, 2013). The existence of satellite DNA depends on the presence of its helper virus, especially in the process of pathogenesis and the severity of symptoms. Thus far, the existence of betasatellite has \(\beta\)C1 gene can increase the severity of symptoms because it can disrupt the synthesis of defense compounds, such as jasmonic acid and disrupt transcriptional gene reduction (Transcriptional Gene Silencing/TGS) (Bhattacarya \textit{et al.}, 2015). The existence of alphasatellite has a gene similar to the C1 begomovirus gene that encodes a replication protein cause a decrease in the severity of symptoms (Idris \textit{et al.}, 2011). Therefore, this research aimed to detect and to characterize the molecular of begomovirus and betasatellite on \textit{A. conyzoides} that shown vein yellowing symptoms and asymptomatic. The results of this study are considered to be the basis for developing a strategy to control the disease caused by Begomovirus infection associated with betasatellite, especially on \textit{A. conyzoides} that act as a virus reservoir.

**MATERIALS AND METHODS**

**Location and Sampling Time**

\textit{A. conyzoides} samples were collected from Magelang, which is an endemic area of begomovirus in September 2018. Samples collected were the weed with no symptom and weed that showed vein yellowing with mosaic symptoms. The samples appearance of symptoms were documented and used for molecular analysis including total DNA extraction, molecular detection of begomovirus and betasatellite using PCR method, nucleotide sequencing, and data analysis.

**DNA Extraction**

The DNA extraction was performed using a total DNA extraction kit for plants (Geneaid, Germany). The extraction was employed based on the supplier instructions. The extracted DNA was used as a template for the detection of begomovirus and betasatellite using PCR.

**Molecular Detection**

The PCR method for begomovirus was performed by universal primer pair of Krusty (5’–CCNMRD GGHTGTGARGGNCC–3’) and Homer (5’–SVD GCRTGVGTRCANGCCAT–3’) which amplified DNA-A genomes with a target of 580bp (Revill \textit{et al.}, 2003). Betasatellite detection was performed by \(\beta\)01 (5’–GGTACCCTACGCTACGCAGCAGCC–3’) and \(\beta\)02 (5’–GGTACCTACCCTCCAGGGGTACAC–3’) specific primers which amplified the intact genome of betasatellite with a target of 1300bp (Briddon \textit{et al.}, 2002). PCR was carried out with a total volume of 50 µl for each sample: 25 µl MyTaq Red Mix (Bioline), 2 µl forward primer, 2 µl reverse primer, 19 µl ddH\textsubscript{2}O, and 2 µl template. The PCR for detecting begomovirus was performed in 35 cycles: initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 1 minute, annealing at 55°C for 30 seconds, elongation at 72°C for 1 minute, and the final elongation at 72°C for 10 minutes. The PCR program for the detection of betasatellite carried out in 35 cycles: initial denaturation at 95°C for 3 minutes, denaturation at 95°C for 1 minute, annealing at 55°C for 30 seconds, elongation at 72°C for 1 minute, and the final elongation at 72°C for 10 minutes. The PCR products was electrophoresed using 1% agarose gel, then visualized by UV light. The PCR products were used in nucleotide sequencing.
**Nucleotide Sequencing**

Nucleotide were sequenced using direct sequencing performed by PT Genetika Science Indonesia.

**Data Analysis**

Nucleotide sequence data were analyzed by MEGA 7 software (Kumar et al., 2016), BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi), ORF Finder program (https://www.ncbi.nlm.nih.gov/orffinder/), and Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Data analysis were conducted including alignment, homology percentages using BLAST, ORF detection, and phylogenetic tree. The phylogenetic tree was conducted using Neighbor Joining (NJ) method with 1000 bootstraps.

**RESULTS AND DISCUSSION**

*A. conyzoides* samples were collected from Magelang, Central Java in 2018. Samples collected are healthy and symptomatic plants (bright yellowing, mosaic, vein chlorosis of leaves) considered to be infected by Begomovirus (Figure 1). Begomovirus detection was performed by the PCR method using a Krusty-Homer primer pair, amplified fragment of the CP begomovirus gene. Betasatellite detection was carried out using specific primer pair β01/β02 amplified betasatellite genome. Amplification with Krusty- Homer primer pair produced amplicon sized approximately 580bp, while amplification with primer pair β01/β02 produced an amplicon sized approximately 1300bp (Figure 2). This result revealed that the sample was infected by begomovirus with a betasatellite.

Direct nucleotide sequencing using specific primers for begomovirus and confirmed by BLAST program showed samples of *A. conyzoides* infected by ToLCJaV, a close relationship with ToLCJaV Ageratum from West Java, Indonesia (AB162141) (Kon et al., 2007) with the homology of 95.5%. Phylogenetic analysis showed that ToLCJaV in Indonesia is positioned in separate cluster compared

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**Figure 1.** Comparison of healthy (left) and symptomatic *Ageratum conyzoides* showed severe leaf yellowing and vein clearing caused by Begomovirus (right)

**Figure 2.** PCR visualization with targets begomovirus of 580 bp and betasatellite of 1300 bp; column 1 and 2 are symptomatic *Ageratum conyzoides*; column 3 and 4 are healthy *A. conyzoides*; M is DNA ladder 100 bp
to ToLCV from India (Figure 3). Besides infecting tomato plants, ToLCJaV and AYVV can infect *A. conyzoides*, which resulted in multiple infections by ToLCJaV and AYVV (Kon *et al.*, 2007). This finding showed that the presence of *A. conyzoides* around tomato plantations may role as an alternative host and viral reservoir for begomovirus.

Direct nucleotide sequencing using the betasatellite primer and confirmed by the BLAST program showed that betasatellite was detected with the homology of 88% related with *Tomato yellow leaf curl betasatellite* (ToLCB) Ageratum isolate from West Java (Kon *et al.*, 2007). This result showed the association with ToLJaV as its helper. Analysis nucleotide sequencing showed the presence of a satellite conservative region (SCR), TAATATTAC stem-loop structure (Figure 4), ORF βC1, and adenine rich region (A–rich region) with adenine content more than 60% (Zhou, 2013) Analysis phylogenetic showed betasatellite samples from Ageratum (in this study) are in the same branch with other betasatellite associated with AYVV and ToLCV. Therefore, there is a close relationship between betasatellite associated with ToLCJaV and betasatellite associated with AYVV and ToLCV (Figure 5). Alignment of ORF βC1 with several betasatellite in the Genbank database showed the presence of ORF along 118 amino acids (Figure 6). This finding similar to the characteristics of betasatellite studied by Kon *et al.* (2007) and Shahid *et al.* (2014).

Betasatellite associated with ToLJaV has the characteristics of TAATATTAC stem-loop structure, the presence of an ORF along 118 amino acids, and the presence of adenine-rich regions. This result was similar to previous studies by Kon *et al.* (2007) and Shahid *et al.* (2014). These characteristics distinguish

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Figure 3. Phylogenetic diagram of ToLCJaV compared to other ToLCV in the Genbank database; samples are marked with a black dot; analysis of nucleotide sequences was conducted by Neighbor Joining 1000 bootstraps

| Sample Name                                      | Genbank Accession | Sequence | Bootstrap |
|-------------------------------------------------|-------------------|----------|-----------|
| AB100304 ToLCJaV Tomato Indonesia               |                   |          |           |
| AB16214 ToLCJaV Ageratum Indonesia             |                   |          |           |
| KU686805 ToLCV Tomato India                     |                   |          |           |
| DO629101 ToLCV Tomato India                     |                   |          |           |
| KP725959 ToLCV Papaya India                    |                   |          |           |

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Figure 4. Stem-loop pattern of betasatellite samples; stem marked with red-colored box, loop marked with green-colored box, and conservative nonanucleotide TAATATTAC marked with blue-colored box
betasatellite from other satellites (alphasatellite and deltasatellite). Alphasatellite has a half size of begomovirus genome, characterized by TAATATTAC stem-loop, an ORF resembles ORF C1 in DNA-A of Begomovirus encodes replication proteins so that alphasatellite can replicate by itself, and adenine-rich regions (Zhou, 2013). Deltasatellite has a half size of betasatellite genome characterized by TAATATTAC stem–loop structure, however, it has no ORF (Zhou, 2013; Lozano et al., 2016).

The presence of A. conyzoides around cultivated fields is important to be monitored and controlled because it can play a role in disrupting the growth of cultivated plants, becoming a reservoir of viruses, and being an alternative host of B. tabaci. The presence of ToLCJaV associated with ToLCB detected in this study may reveal other risks besides vector populations and begomovirus, i.e. the presence of betasatellite that increases the severity of symptoms due to Begomovirus infection, because betasatellite can be transmitted along with begomovirus by B. tabaci.

CONCLUSION

Based on the nucleotide analysis, the virus infected A. conyzoides that showed bright yellow symptomatic is ToLCJaV, and the betasatellite associated with ToLCJaV is ToLCB. In asymptomatic A. conyzoides, the virus or beta-satellite were not detected.
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