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Research Article

Detection of *Strawberry vein banding virus* (SVBV) and Identification of Viruliferous Insects Associated with Strawberry Plants (*Fragaria* sp.) in Bali

I Putu Sudiarta [1)]*, Gusti Ngurah Alit Susanta Wirya[1)], Dewa Gede Wiryangga Selangga[2)], & Made Getas Pudak Wangi[1)]

[1)]*Faculty of Agriculture, Udayana University*
Jln. P.B. Sudirman, Denpasar, Bali 80234 Indonesia

[2)]*Faculty of Agriculture, Warmadewa University*
Jln. Terompong No. 24, Tanjung Bungkak, Denpasar, Bali 80239 Indonesia

**Corresponding author. E-mail: putusudiarta@unud.ac.id*

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ABSTRACT

Plant pests and diseases cause decreases in strawberry yield in Bali, including plant viruses. *Strawberry vein banding virus* (SVBV) is a virus that infects strawberry plants and is transmitted by insects. However, studies about this disease in Bali are still limited. This study aimed to detect SVBV within insect bodies and determine the insect species of its vector. Methods used included (1) sampling; (2) detection of SVBV from insect bodies using primers (SVBV F/SVBV R) and PCR; and (3) molecular identification of viruliferous insect with primers (LCO 1490 F/HCO 2198 R) using PCR and sequencing analysis. This study successfully detected SVBV DNA in an insect from three insect families associated with strawberry plants in Pancasari Village, Buleleng Regency, Bali. Results from the nucleotide sequences analysis in SVBV viruliferous insects indicate that the insect was *Myzus persicae*.

Keywords*: Myzus persicae*; strawberry; *Strawberry vein banding virus* (SVBV); viruliferous insect

INTRODUCTION

Strawberry vein banding virus (SVBV) is one of the viruses that infect strawberry plants and is a member of the genus Caulimovirus, Caulimoviridae (Pattanaik *et al*., 2004). This virus was first reported in America in 1955 and is currently present in Europe, North America, Australia, Brazil, and Japan (Chen *et al*., 2016; Feng *et al*., 2016). In recent years, SVBV has also been reported to cause symptoms on strawberries in several provinces in China (Feng *et al*., 2016; Stenger *et al*., 1988). Symptoms caused by SVBV, in general, are vein banding of the leaf bone, necrosis around the leaf bones leaf and leaves slightly curling (Martin & Tzanetakis, 2006). Insects are one of the agents that can transmit this virus to healthy plants, and some insects that are vectors of viruses can act as mediums for virus replications. Vector insects are known to belong to the Aphididae, Auchenorrhyncha, Aleyrodidae, or Thysanoptera groups (Martin & Tzanetakis, 2006).

Although there have been no reports of this disease in Bali, results from Yulianingsih's research in 2020 successfully detected this virus from strawberry plants with symptoms of SVBV infections from Pancasari Village, Buleleng Regency, Bali using PCR. This research was conducted to detect SVBV DNA in the bodies of insect species associated with strawberry plants, suspected as an SVBV vector insect, and molecular identification was made. This knowledge can be used as a basis for management methods to maintain strawberry production by controlling insect vectors.

MATERIALS AND METHODS

Field Survey and Samples Collection

Field survey and sample collections were conducted in October 2019 at several strawberry growing areas at Pancasari Village, Buleleng Regency, Bali. Field sampling was carried out by collecting insect imagoes (whitefly, aphid, or thrips) on strawberry plants infected by SVBV using an insect aspirator. Insect samples were then placed in Eppendorf tubes.

Detection of SVBV in Insect

DNA extraction was done using the CTAB method (Doyle & Doyle, 1990). Imago insect extractions were done in Eppendorf tubes and liquid nitrogen and extraction buffer solution were added (EDTA-20 mM, Tris-HCl, pH 8-100 mM, NaCl-1.4 M, CTAB-2%, and Mercaptoethanol-0.2%). Solutions were incubated at 65°C for 60 minutes, occasionally mixing by gently inverting the tubes. After incubation, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) solution was added, and the tubes were inverted several times, followed by centrifugation at 12,000 rpm for 15 minutes. The upper phase was transferred to a new microtube followed by adding sodium acetate (1/10×volume) and cold isopropanol (2/3× volume), then the mixture was incubated overnight at -20°C to precipitate DNA. After the incubation, the tube was centrifuged at 12,000 rpm for 10 minutes, and the supernatant was removed. The pellet containing total DNA was washed with 96% ethanol and centrifuged at 8,000 rpm for 5 minutes. DNA pellets were air-dried and resuspended in TE buffer solution (1x) and stored at -80°C for further use.

The amplification reaction used the PCR. For each reaction, 2 µl of DNA, 1 µM of each SVBV F/R primer, 0.5 µl of $MgCl_2$, 12.5 µl of *Dream taq green master mix,* and distilled water was added to the final solution until it reached a volume of 25 µl and placed in a PCR bead. DNA amplification was done in a thermal cycler (Gene Amp, PCR System 9700 PE Applied Bio-system) started with a pre-heating cycle for 2 minutes at 94°C, followed by 40 cycles of denaturation (120 seconds at 94°C), annealing (30 seconds at 52°C), and extension (60 seconds at 72°C). The last cycle ended at 72°C for 10 minutes and cooled down to 4°C. The amplicon was then visualized with electrophoresis using 1% agarose gel in 0.5× TBE (Tris-Boric acid-EDTA) buffer. The electrophoresis was performed at 100 V for 30 minutes and visualized under a UV transilluminator.

Molecular Identification of Viruliferous Insects

DNA extraction was carried out using the CTAB method (Doyle & Doyle, 1990). The DNA extraction method was similar to the detection of SVBV in the insect method. DNA of viruliferous insects were amplified using PCR with universal primers LCO 1490 (5'GGTCAACAAATCATAAAG ATATTGG3') and HCO 2198 (5'TAAACTTCA GGGTGACCAAAAAATCA 3'). The amplicon was then visualized by electrophoresis using 1% agarose gel in 0.5× TBE (Tris-Boric acid-EDTA) buffer. The electrophoresis was performed at 100 V for 30 minutes and visualized under a UV transilluminator.

Sequence and Phylogenetic Analysis

PCR product was subjected to direct sequencing, and the sequence data were analyzed using BioEdit V.7.0.5, CLC Sequence Viewer 8, and MEGA 6.06.

RESULTS AND DISCUSSIONS

Strawberry plants infected with the SVBV in Pancasari Village, Buleleng Regency, Bali showed darker leaf bone colors than the leaf lamina, and the leaves showed chlorosis (Figure 1A and B). Viral infection further causes the plant to experience malformations, such as stunting and leaves shrinkage. These symptoms were similar to those found on strawberry plants in California (Dara, 2015).

Insect samples collected from strawberry growing areas in Pancasari Village, Buleleng, Bali were amplified using SVBV F/R primers. PCR amplification was performed to detect SVBV in bodies of insect species associated with strawberry plants in Pancasari Village, Buleleng Regency, Bali. The seven insect samples in this experiment were collected from the strawberry growing areas in the Village of Pancasari, Buleleng, Bali (A1, A2, A3, A4, A5, A6, and A7); A7 was the only sample detected to contain SVBV positively. Amplification with forward and reverse SVBV successfully detected

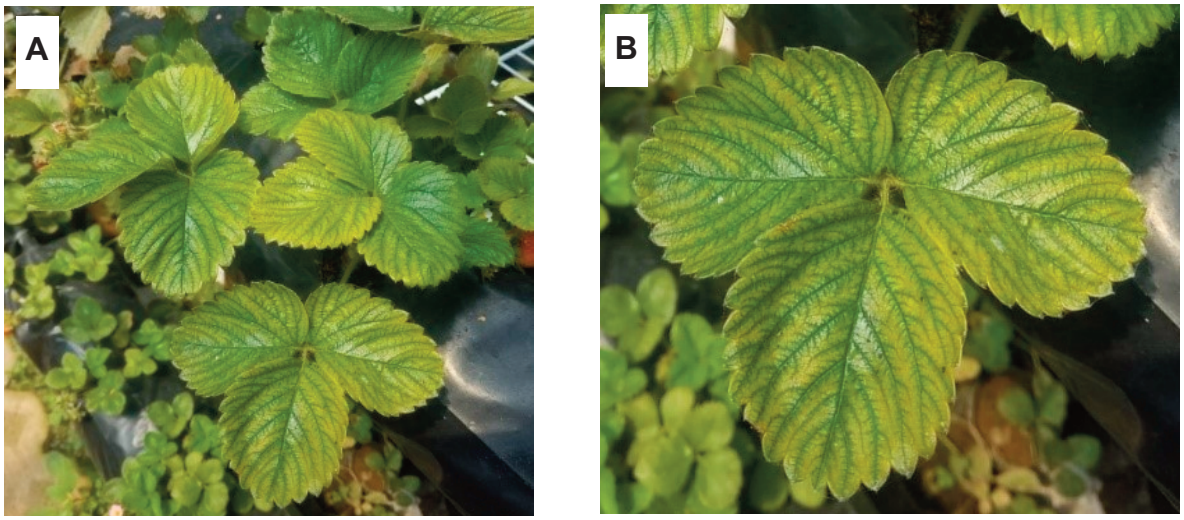

Figure 1. Chlorotic vein banding symptoms caused by *Strawberry vein banding virus* found in Pancasari Village, Buleleng Regency, Bali; (A) symptoms of chlorotic vein banding, (B) enlargement of symptoms of chlorotic vein banding

Figure 2. DNA visualization of *Strawberry vein banding virus* using PCR from insect samples associated with strawberry plants in Pancasari Village, Buleleng Regency, Bali (M: Marker; A1 and A2: whitefly; A3 and A4: thrips; A5, A6, and A7: aphid)

SVBV DNA in an insect's body with a baseband measuring ± 472 bp (Figure 2). Based on morphological identifications, samples A1 and A2 were whiteflies; A3 and A4 were thrips, while A5, A6, and A7 were aphids. Insects that contained SVBV DNA were then identified molecularly to determine insect species.

Viruliferous insects were molecularly identified to know the insect species through DNA fragments of MtCOI. Insect identification was done by PCR amplification using forward LCO 1490 primers and reverse HCO 2198 primers. DNA band measuring ± 650 bp was successfully amplified with its primer on DNA from the A7 sample (Figure 3). PCR products from viruliferous insects were then sent to PT Genetic Science Indonesia to trace nucleotide sequences to determine the relationship between kinship and homology with various DNA sequences in GenBank (NCBI program).

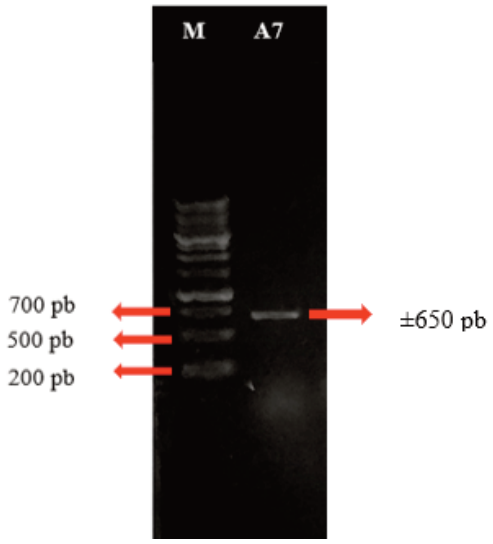

Figure 3. DNA visualization of A7 viruliferous insect using PCR with LCO 1490 forward primer and HCO 2198 reverse primer. (M: Marker; A7: viruliferous insect DNA)

Sequence and Phylogenetic Analysis

Sequence results of insect DNA sample were compared with the species *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) insect MtCOI sequences in the NCBI database, namely GU 568500 (France), KC 286663 (China), KR 035299 (Canada), KX 631542 (South Korea), MF 140519 (India), MH 543312 (Bangladesh), FN 868601 (United Kingdom), KY 323042 (Kenya), MH 183022 (Iran), KY 634530 (New Zealand) and *Aphis gossypii* (Glover) _JQ 067101 (India) that were chosen for nucleotide sequence analysis.

Sequence analysis results of insect samples (A7_BALI) showed 100% similarities to insect samples from France, Canada, South Korea, Iran, Kenya, Bangladesh, India, China, and the United Kingdom. Based on the analysis of insect kinship, the insect species was *Myzus persicae* (Hemiptera: Aphididae). *Aphis gossypii* insect sequence originating from India used as a comparative outgroup showed 90% sequence homology.

The phylogeny was done from the sequence results, then entered into Clustal X and MEGA 6.06. The phylogenetic results are shown in Figure 4. *Myzus persicae* have been reported to be able to transmit various types of virus, one of which is *Potato Virus Y* (PVY) on potato plants (Ruimassa *et al.*, 2003).

The Morphological Description of *Myzus persicae*

Aphids are polyphagous pests that are very common in plants. There are many species of aphids; one of them is *Myzus persicae*. In the field, *M. persicae* has similarities with other types of aphids, such as *Aphis gossypii*; therefore, it is difficult to distinguish without a microscope. In this study, *M. persicae* was found in strawberry plantations was dark green in color with a body like a pear. The key that is often used to identify morphologically is the presence of the tubercle and cornicle of the aphids. *M. persicae* was found to have a developed tubercle (Figure 5a). The *M. persicae* has a pair of bulges on the end of the abdomen called a cornicle (Figure 5b). Compared with *A. gossypii*, *M. persicae* has a longer cornicle (Figure 5b) (Choi *et al.*, 2019).

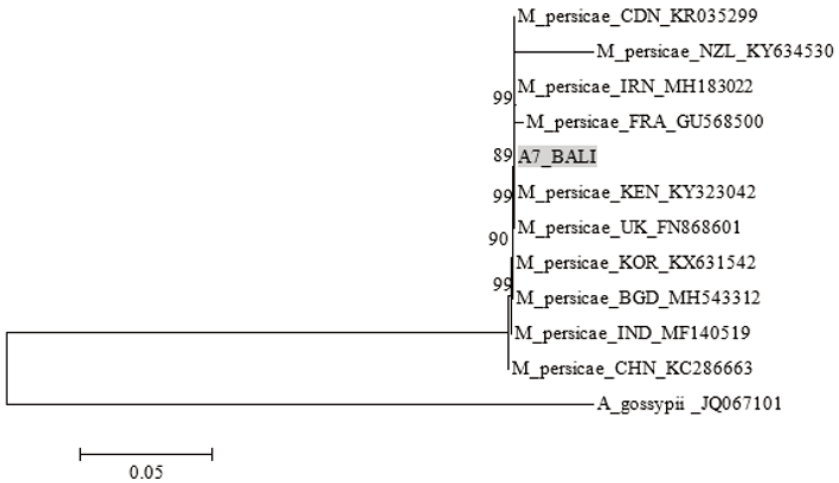

Figure 4. Phylogenetics of aphid insect (viruliferous insect) found from strawberry planting areas in Pancasari Village, Buleleng Regency, Bali

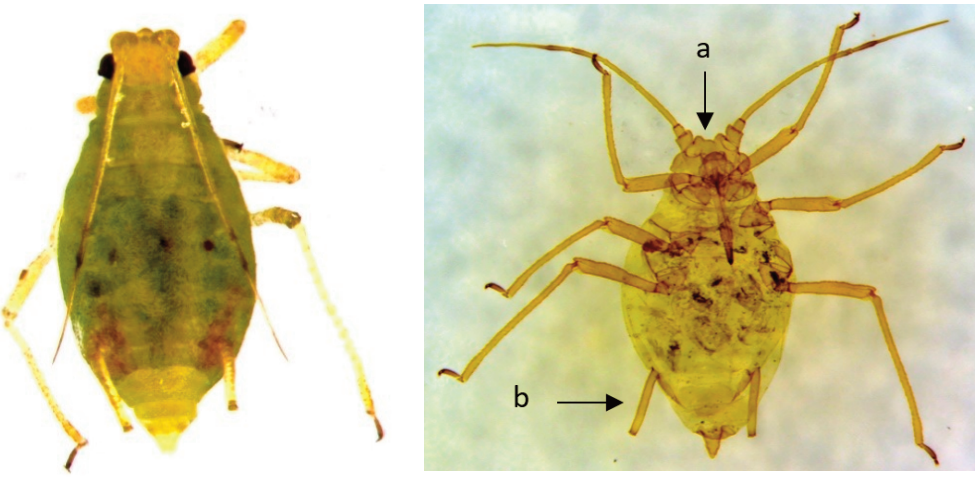

Figure 5. The morphological description of *Myzus persicae*; (a) the tubercle *M. persicae*, (b) cornicle of *M. persicae*

CONCLUSION

This research successfully detected SVBV DNA in one insect (aphid) from three insect families associated with strawberry plants in Pancasari Village, Buleleng Regency, Bali. The results of nucleotide MtCOI sequences analysis of viruliferous vector insects of SVBV indicate it was *Myzus persicae*. These results are similar to those reported by several researchers that aphids are potential vectors of SVBV (Frazier & Converse, 1980; Vašková *et al.*, 2004).

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