

RESEARCH PAPER

## Symptoms variation and molecular characterization of *Strawberry vein banding virus* in Bali, Indonesia

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### ABSTRACT

Vein banding disease has affected young strawberry leaves in Bali over the past five years. Caused by *Strawberry vein banding virus* (SVBV), the disease is primarily associated with fruit size reduction, though its exact impact remains unclear. This study aims to assess symptom variation, disease severity, and the molecular characteristics of SVBV in Bali strawberry plants. Field observations and molecular identification were conducted using PCR with SVBV-specific primers targeting the CP gene. Samples were collected from ten locations in Bali, including Pancasari, Candikuning, Wanagiri, Gobleg, and Kembang Merta. SVBV DNA from Candikuning, Pancasari, and Kembang Merta was successfully amplified, confirming SVBV infection as the cause of vein banding symptoms. SVBV induces vein banding with upward and downward leaf curling. Disease incidence was highest in Pancasari 1 and 3 (80%) and lowest in Wanagiri 1 and Gobleg (20–28%). Disease severity ranged from 13% to 83%, with the highest recorded in Pancasari, Buleleng, and the lowest in Kembang Merta, Tabanan. Molecular analysis revealed that the SVBV isolate from Bali shares 99.3–100% sequence homology with Chilean isolates. Phylogenetic analysis showed clustering with SVBV isolates from the United States, Brazil, and Chile. This study provides the first molecular characterization of SVBV in Bali, contributing to a better understanding of its epidemiology and potential impact on strawberry production.

**Key words:** Caulimovirus, disease severity, PCR, SVBV

### INTRODUCTION

Strawberry (*Fragaria* sp.) is one of the horticultural crops developed in Indonesia. This popular fruit has various health benefits and a high economic value. It was first discovered in Chile, America, and its geographical distribution later extended to the Americas, Europe, and Asia, including Indonesia (Edger et al., 2019).

Strawberry cultivation in Indonesia initially began in North Sumatra, East Java, West Java, Central Java, and Bali. In Bali, strawberry farming has been developed in the villages of Pancasari, Kembang Merta, and Candikuning.

Indonesian strawberry production has been unable to meet market demand. In 2015, production reached 31,801 tons. In 2016, it dropped to 12,091 tons, and in 2017, it slightly increased to 12,225 tons. However, production declined again to 8,541

tons in 2018 (Central Bureau of Statistics, 2019). Poor cultivation practices and the presence of plant-disturbing organisms (PDOs), including viruses, have contributed to this decrease.

*Strawberry vein banding virus* (SVBV) causes severe injury to strawberry plants. It belongs to the genus *Caulimovirus* and the family *Caulimoviridae*. The circular DNA of SVBV is 7.8 kb in length (Yang et al., 2022).

SVBV has been reported to infect strawberries in North and South America, Europe, Africa, Australia, Japan, and China (Li et al., 2018; Ren et al., 2022; Yang et al., 2022). The virus was first detected in strawberry plants in the United States in 1955 and has since spread to Europe, Australia, Brazil, North America, and Japan (Jiang et al., 2021; Yang et al., 2022). In recent years, SVBV has also been found in several Chinese provinces (Jiang et al., 2021).

SVBV is transmitted by the insect vector *Chaetosiphon fragaefolii* through semi-persistent transmission or plant grafting (Yang et al., 2022). Infected plants typically exhibit vein thickening, necrosis around the veins, and minor leaf curling (Martin & Tzanetakis, 2013). Common SVBV symptoms observed in the field include vein banding,

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leaf curling, stunting, and necrosis (Ren et al., 2022).

SVBV has been detected in strawberry plants in Pancasari Village, Buleleng Regency, Bali, using the PCR method (Yulianingsih, 2020). Among plant viruses, SVBV has been reported to infect strawberries in Indonesia and is associated with viruliferous insects (Sudiarta et al., 2021).

Strawberry plants infected with SVBV in Pancasari Village exhibited darker veins compared to the leaf lamina, along with chlorosis leaves. Advanced viral infection can cause malformations such as stunting and shriveling of the plant foliage. These symptoms are identical to those observed in California strawberry plants infected with SVBV (Tzanetakis & Martin, 2014).

This study was conducted due to limited information on disease severity caused by SVBV infection and aimed to determine the molecular characterization of the SVBV isolate in Bali, Indonesia. The incidence and severity of SVBV disease are essential for assessing the level of infection and its spread in the field.

The molecular characteristics of the SVBV isolate from Bali is crucial for developing field-based control strategies and SVBV-resistant varieties. Additionally, the development of an appropriate diagnostic tool for SVBV detection in strawberries can aid in identifying virus-resistant cultivars.

## MATERIALS AND METHODS

**Research Site.** This study applied a purposive sampling method for collecting strawberry leaves from several location in Bali Buleleng and Tabanan Regency. Identification was carried out at the Plant Protection Laboratory of the Udayana University, Denpasar, from February 2022 to January 2023.

**Survey and Sampling.** The survey and sampling were conducted in 10 strawberry cultivation areas in Buleleng Regency (Pancasari 1, Pancasari 2, Pancasari 3, Wanagiri 1, Gobleg 1 Villages) and Tabanan Regency (Candikuning 1, Candikuning 2, Candikuning 3, Kembang Merta 1, Kembang Merta 2 Villages). A total of 50 samples were taken from each location, resulting in 500 samples analyzed.

Plant samples at each observation site were determined using the diagonal method. Plants exhibiting SVBV symptoms, as well as asymptomatic plants, were selected for leaf collection. Leaf samples were used to confirm SVBV infection through PCR detection. Ten symptomatic leaf samples were collected

from each field. The leaves were placed in a cooler and transported to the laboratory. Approximately 0.1 g of each leaf sample was weighed and stored in a freezer at -20 °C for further use in the virus detection stage.

**Observation of Disease Incidence and Severity in the Field.** Disease symptoms were observed on yellowing or mosaic-patterned leaves of strawberry plants. Disease incidence (IP) was measured using the following formula:

$$DI = \left( \frac{n}{N} \right) \times 100\%$$

- DI = Disease incidence (%);  
n = Number of symptomatic plants;  
N = Total plants of plants observed.

The severity of the disease was calculated based on the proportion of infected plants in the population using the following formula:

$$DS = \left( \frac{\sum_{i=1}^n n_i \times v_i}{N \times Z} \right) \times 100\%$$

- DS = Disease severity (%);  
n = Number of plants assigned a spesific score;  
v = Score assigned to infected strawberry plants;  
N = Highest score value;  
Z = Total number of strawberry plants observed.

Disease severity was assessed by evaluating the variety of visual symptoms observed in the field and assigning a numerical score (0–5) to each symptom.

- 0 = No symptoms;  
1 = Leaf chlorosis;  
2 = Leaf vein banding;  
3 = Leaf vein banding and mosaic;  
4 = Leaf vein banding, green streak, and chlorosis;  
5 = Leaf vein banding, green streak, chlorosis, and plants stunting

**Detection of SVBV by PCR.** The PCR method used leaf samples from the field for SVBV detection. Total DNA extraction from strawberry plants was performed using the CTAB (cetyltrimethyl ammonium bromide) method. Symptomatic strawberry leaves (0.1 g) were ground with liquid nitrogen. Once the sample was finely crushed, 500 µL of CTAB buffer was added using a micropipette. The sample was then transferred to a

new tube, heated in an oven, and shaken at 65 °C for 1 h.

Next, the sample was incubated for 2 min, and 500 µL of C:I buffer (chloroform:isoamyl alcohol) was added. The tube was inverted for 5 min and centrifuged at 12,000 rpm for 15 min to separate the supernatant. The supernatant was transferred to a new tube, and sodium acetate (CH<sub>3</sub>COONa) was added at a volume of 1/10 of the supernatant. The tube was inverted again for 5 min. The supernatant was then discarded, and 2/3 of the total volume of isopropanol was added. The sample was incubated overnight and stored in a freezer at -20 °C. The tube was centrifuged again at 12,000 rpm for 10 min. The liquid was discarded, and the precipitate was washed with 70% ethanol. The tube was centrifuged again at 8,000 rpm for 5 min, after which the liquid was removed. The precipitate was dried by placing the tube upside down on a tissue for 1 hour. Finally, 200 µL of nuclease-free water was added. The extracted DNA was used as a template in the amplification stage using the PCR technique.

Viral DNA was amplified using specific primers: SVBV(F)(5'-TGAACGCAAAAATCCTATC-3') and SVBV (R) (5'-TGTTCTGAACAGATTGAATC-3'), which targeted the coat protein (CP) gene with an expected amplicon size of approximately 472 bp (Mahmoudpour, 2003).

The composition of the amplification reaction was as follows: 1 µL DNA template, 1 µL SVBV F 1 primer, 1 µL SVBV R 2 primer, 9.5 µL nuclease-free water, and 12.5 µL Green Taq DNA Polymerase. The DNA amplification program included: predenaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 52 °C for 1 min, DNA strands synthesis at 72 °C for 1 min, and final elongation at 72 °C for 7 min, with storage at 4 °C.

The amplification results were analyzed by electrophoresis using a 1% agarose gel in 1× TAE buffer (Tris-Acetate-EDTA) at 50 volts for 50 min. The gel was then visualized using a gel documentation system.

**Sequencing Analysis.** The target viral DNA fragment obtained from PCR amplification was sent to First Base, Malaysia, for sequencing. Nucleotide and amino acid sequence identify was analyzed using BioEdit V.7.0.5 software, while the phylogenetic tree was constructed using MEGA 11 software (Tamura et al., 2021).

## RESULTS AND DISCUSSION

During the field survey, strawberry plants exhibited symptoms typical of viral infection, including leaf vein banding, leaf curling, and stunting. However, some plants remained asymptomatic (Figure 1). To detect the presence of SVBV in strawberry plants, 50 samples were collected from various regions of Bali, analyzed by PCR, and sequenced using SVBV-specific primers. According to Ren et al. (2022), symptoms of SVBV infection in leaves include mottling, stunting, and clustering, although many infected plants remain asymptomatic.

The symptoms caused by infection with different virus strains within the same species, as well as in various host plants, can range from asymptomatic to severe mosaic symptoms (Selangga et al., 2022). Viral infection in host plants leads to a reduction in chlorophyll a, chlorophyll b, carotenoids, carbohydrates, proteins, and amino acids. Variations in the degree of reduction in these components contribute to differences in disease severity scores (Jabeen et al., 2017; Soni et al., 2022).

The highest disease incidence, reaching 80%, was observed in Pancasari 1 and Pancasari 3. In contrast, the lowest incidence, ranging from 20% to 28%, was recorded in Wanagiri 1 and Gobleg 1 (Table 1). Disease severity in strawberry plants ranged from 13% to 83%, with Pancasari, Buleleng exhibited the highest disease severity, while Kembang Merta, Tabanan showed the lowest (Table 1). The Rosa Linda and Sweet Charlie strawberry cultivars were found to be susceptible to SVBV infection. Furthermore, cultivation techniques significantly influenced disease severity in strawberry

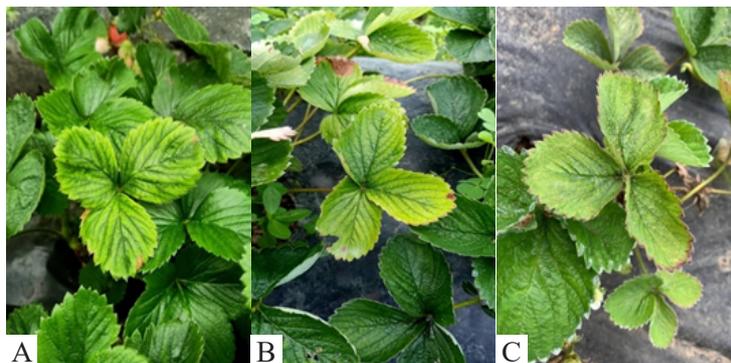


Figure 1. The symptoms variation of SVBV in field. A. Mosaic, vein banding; B. Chlorosis in lamina; C. Chlorosis.

plants in Bali. Good cultivation practices generally resulted in lower disease severity, while poor practices facilitated rapid disease spread.

The primers used for the PCR assay were designed based on highly conserved regions of the coat protein (CP) genes in the SVBV genome. These primers were initially developed in 2000 to detect the American SVBV strain. After more than two decades, they remain effective for detecting most global SVBV isolates. The brightness of all amplified bands was consistent, with no weak bands observed, aligning with previous findings (Li et al., 2018). SVBV DNA from Candikuning, Pancasari, and Kembang Merta was successfully amplified using SVBV-specific primers, targeting the  $\pm 472$  bp CP gene (Figure 2). This confirms that the vein banding symptoms observed in young strawberry leaves were caused by SVBV infection. SVBV induces various symptoms, including vein banding and upward or downward leaf curling.

The nucleotide sequence homology of the 10 isolates from Bali compared with isolates from other countries ranged from 80.3% to 100% (Table 2). The Bali SVBV isolates showed the highest homology (99.3% and 100%) with isolates from Chile, specifically those with accession numbers JN542478 and JN542479,

respectively. In contrast, the lowest homology (80.3%) was observed with an isolate from China.

To elucidate the relationships among SVBV isolates, a phylogenetic tree was constructed based on available CP gene sequences. The tree revealed that SVBV isolates from Bali, Indonesia, and other countries clustered into seven distinct groups (Figure 3).

Group 1: isolates from the United States, Brazil, Chile, and Indonesia.

Group 2: isolates from China.

Groups 3, 4, and 5: Chilean isolates (JN542474, JN542476, JN542480),

Groups 6 and 7: Chinese isolates (AY862389 and KJ774105).

The phylogenetic tree indicates that SVBV isolates from Bali, Indonesia, form a subgroup with the Chilean isolates (JN542478 and JN542479). According to Ren et al. (2022), the evolution of SVBV is closely linked to its geographic distribution, as demonstrated by the clustering patterns of the CP gene phylogenetic tree and the overall SVBV genome.

Variations in symptoms may result from the type of virus infecting the plant, as well as environmental factors (Listihani et al., 2020; Listihani et al., 2022a; Listihani et al., 2022b; Selangga & Listihani, 2022;

Table 1. Disease incidence, severity and field symptoms of SVBV infecting strawberry in Bali, Indonesia

Location	Cultivar	Symptoms variation	Disease incidence (%)	Disease severity (%)	PCR detection
Pancasari I, Buleleng	Rosa Linda	Mosaic, vein banding	80	83	+
Candikuning I, Tabanan	Sweet Charlie	Mosaic, vein banding	70	52	+
Wanagiri I, Buleleng	Rosa Linda	Chlorosis	20	18	+
Gobleg I, Buleleng	Rosa Linda	Chlorosis	28	13	+
Kembang Merta I, Tabanan	Sweet Charlie	Mosaic, vein banding	50	47	+
Pancasari II, Buleleng	Rosa Linda	Mosaic, vein banding	70	61	+
Candikuning II, Tabanan	Rosa Linda	Green streak, chlorosis	70	55	+
Kembang Merta II, Tabanan	Rosa Linda	Chlorosis	50	21	+
Pancasari III, Buleleng	Sweet Charlie	Chlorosis on lamina	80	71	+
Candikuning III, Tabanan	Rosa Linda	Chlorosis on lamina	60	35	+

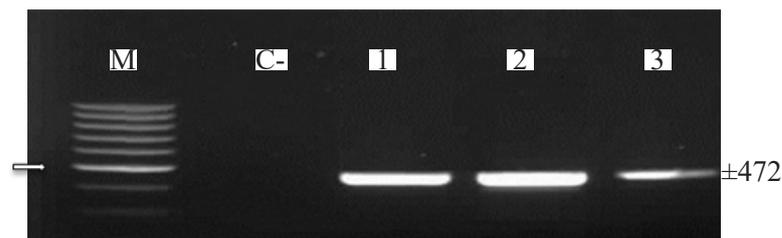


Figure 2. Visualization of DNA amplification of SVBV from leaf samples using primers for SVBV (SVBV-F/SVBV-R) on 1% gel agarose; isolate from Bali. M= DNA marker (1 kb ladder); C-= negative control; 1= Candikuning; 2= Pancasari; 3= Kembang Merta.



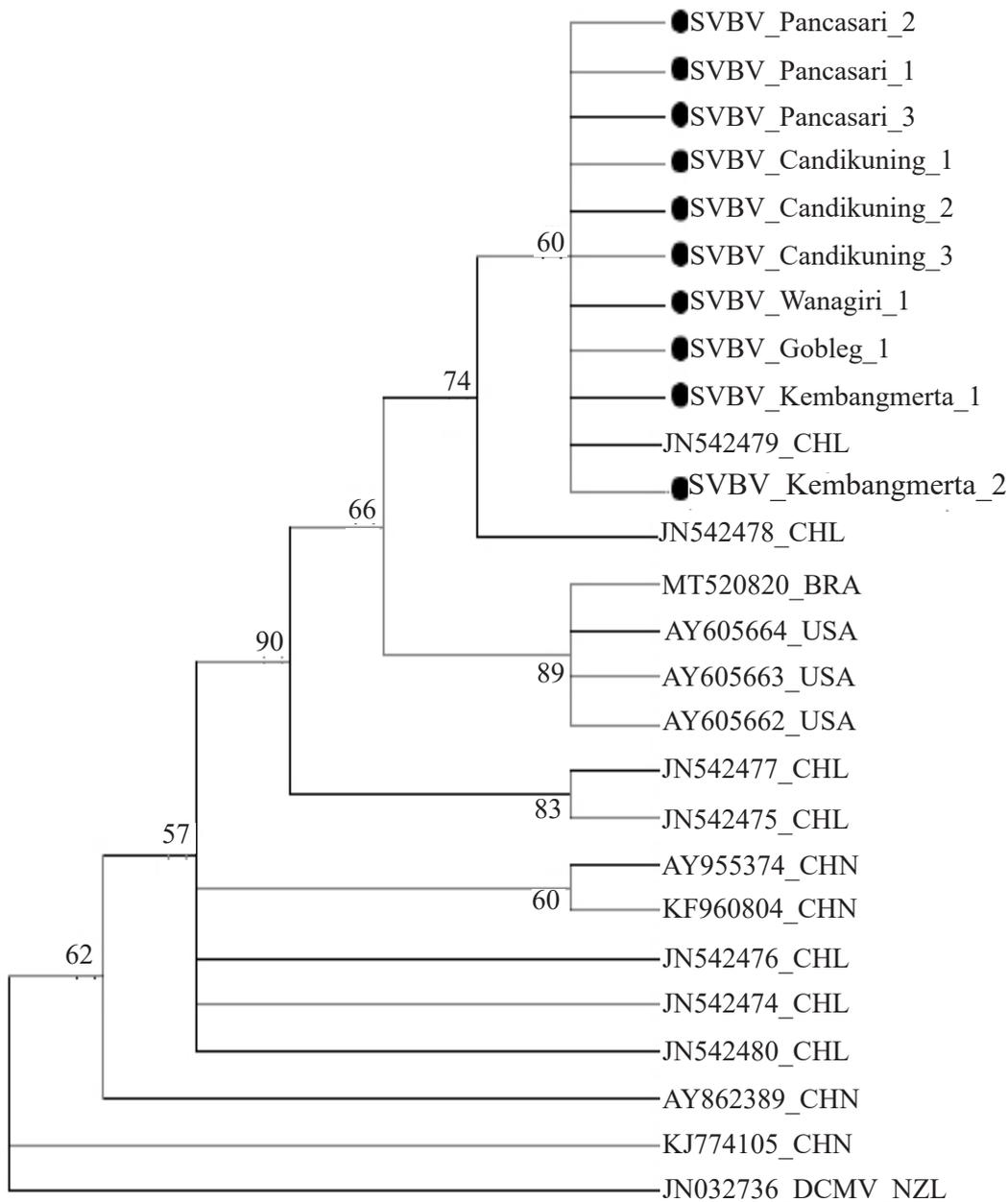


Figure 3. Phylogenetic trees of full-length CP SVBV nucleotide sequences of the DNA-S were constructed using Mega 6.06 with the Neighbor-Joining algorithm and 1000 bootstraps replicate. SVBV isolates from Bali were compared against SVBV isolates from GeneBank. *Dahlia common mosaic virus* (DCMV) was used as an outgroup. Isolates labeled in parentheses ( ) represent those from Bali (SVBV\_Bali) and include isolates from Indonesia (IDN), the United States (USA), Brazil (BRA), Chili (CHL), and China (CHN).

polymerase chain reaction (PCR)—is crucial for virus indexing, rather than relying solely on visual observation (Ding et al., 2019).

SVBV is challenging to detect, and the lack of commercially available antibodies makes serological detection difficult. Additionally, the bioassay technique utilizing indicator plants is time-consuming (Tzanetakis & Martin, 2014). Biological investigations of SVBV and other viruses infecting strawberries are

also hindered by the properties of the host plant, which prevent SVBV transmission through sap (Yang et al., 2022). Furthermore, there are no known alternative hosts for SVBV that could facilitate biological studies. Consequently, obtaining sufficient quantities of purified virus for molecular characterization of the viral genome and virion proteins, as well as for producing specific antibodies, remains constrained by the complexities of strawberry tissue (Ren et al., 2021). For SVBV detection

in Bali, molecular techniques that target specific viral nucleic acids are the most suitable option.

It is essential to evaluate the role of various epidemiological factors in the natural spread of this disease. Resistance genotyping, vector management, and the identification of alternative or primary hosts will be crucial for effective disease control. Based on PCR and viral sequence analysis, it is concluded that SVBV-IDN Bali isolates obtained from diseased strawberry plants are closely related to Asian and American isolates. This study represents the first report on the molecular characterization of SVBV-IDN from Indonesia.

Several SVBV vectors have been identified, including *Acyrtosiphon pelargonii*, *Amphorophora rubi*, *A. idaei*, *Aphis idaei*, *A. rubifolii*, *Aulacorthum solani*, *Chaetosiphon fragaefolii*, *C. jacobii*, *C. tetraerhodum*, *C. thomasi*, *Macrosiphum rosae*, *M. pelargonii*, *Myzus ascalonicus*, *M. ornatus*, and *M. persicae* (Koloniuk et al., 2022; Yang et al., 2022). Among these, *Chaetosiphon* species are the most effective vectors for transmitting SVBV. *Chaetosiphon* is a key vector, particularly when it is abundant, as it frequently transmits the virus from plant to plant. Aphids can acquire and transmit the virus within 30 to 120 min, but vector persistence is typically less than eight hours (semi-persistent transmission). Additionally, certain insect vector species can only transmit specific SVBV strains. SVBV has only been observed infecting *Fragaria* species, with its primary host being wild strawberry (*F. vesca*). While, commercial strawberries can also be infected, symptoms generally appear only under environmental conditions that favor disease development.

Host response and virus-host interaction are widely studied topics in plant virology. Host responses following viral infection and virus-induced defense mechanisms in plants have been extensively reviewed. After infecting plants, viruses must evade plant-initiated defense mechanisms and begin replication to complete their life cycle (Ray & Castell, 2022). In response to infection, plants deploy antiviral immune responses, hypersensitive and necrotic resistance pathways, systemic necrosis, salicylic acid pathways, and R gene-mediated responses (Abebe et al., 2021). A recent transcriptomic study of SVBV-infected *F. vesca* revealed that SVBV influences plant pigment (anthocyanin and flavonoid) metabolism, photosynthesis, and plant-pathogen interactions (Chen et al., 2018).

Strawberry production per unit area has gradually increased in recent years due to improved cultivation

methods, including the use of rented shelters and greenhouses. However, viral vectors play a significant role in spreading the virus, leading to reduced strawberry yields. According to previous reports, severe SMOV infection in combination with SVBV or Strawberry necrotic shock virus (SNSV) can reduce fruit yield by up to 30%, with mixed infections causing even greater losses (Tzanetakis & Martin, 2014). The fruit yield of single plants infected with SMOV and SVBV was 25.5% lower than that of control plants (Silva-Rosales et al., 2013). SVBV infection induces leaf cell death by triggering reactive oxygen species (ROS) accumulation and reducing photosynthesis (resulting in decreased chlorophyll content, stomatal openings, and anthocyanin levels). Viruses also increase abscisic acid (ABA) levels, which can inhibit flowering and contribute to pollen sterility. Furthermore, strawberry virus infections negatively impact fruit quality by increasing fruit hardness while decreasing total soluble solids (TSS) and titratable acidity (TA). These findings are crucial for developing improved control strategies for strawberry cultivation in Bali.

## CONCLUSION

The conclusion of this research is that SVBV has infected strawberry plants in several locations in Bali, including Candikuning, Pancasari, and Kembang Merta. Young strawberry leaves exhibit SVBV infection symptoms, particularly vein banding. The highest disease incidence was recorded found in Pancasari 1 and 3, with an incidence rate of 80%, while the lowest incidence occurred in Wanagiri 1 and Gobleg, ranging from 20% to 28%. Disease severity in strawberry plants ranges from 13% to 83%. The nucleotide sequence homology of the SVBV isolate from Bali, Indonesia, was the highest with isolates reported from Chile (99.3% and 100%). Phylogenetic analysis indicates that the SVBV isolate from Bali clusters with isolates from the United States, Brazil, and Chile.

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## AUTHORS' CONTRIBUTIONS

GNAS, TAP, IPS, DGWS, and SMD conceptualized and designed the experiment. GNAS, DGWS, SMD, and LL conducted the survey and collected strawberry plant samples. GNAS, DGWS, SMD, and LL performed molecular analyses. GNAS, TAP, IPS, DGWS, LL, and SMD contributed to manuscript preparation. The authors provided feedback on the research process, data analysis, interpretation, and manuscript structure. All authors have read and approved the final manuscript.

## COMPETING INTEREST

The authors declare no competing interests, whether financial or non-financial, professional or personal, that could influence the research or its publication.

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