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Detection and Response of Sugarcane against the Infection of *Sugarcane Mosaic Virus* (SCMV) in Indonesia

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Abstract: *Sugarcane mosaic virus* (SCMV) is one among many viruses that infect sugarcane, cause yield loss, and become serious disease agents on sugarcane plantations. Since the morphological symptoms of SCMV are similar to other symptoms caused by *Sugarcane streak mosaic virus* (SCSMV) or nitrogen deficiency, the detection of SCMV is important through accurate diagnostic-like ELISA or RT-PCR. This research aimed to study the causative mosaic pathogen of SCMV in East Java, Indonesia, including mosaic development. The results showed that the mosaic symptom is present in all sugarcane plantations with 78% and 65% disease incidence and severity, respectively. Moreover, the detection procedure based on an amplification of cDNA of the coat protein gene sequence confirmed that SCMV was the causative agent of mosaic disease on sugarcane. Re-inoculation of healthy sugarcane plants with plant sap from a symptomatic leaf from the field showed similar mosaic or yellowish chlorotic areas on the leaf blade, and appeared on the fourth leaves upward from the inoculation leaf, in addition to showing different levels of peroxidase but not total phenol. Mosaic also correlated with the amount of total chlorophyll. Although *Sucrose phosphate synthase* (SPS) protein accumulation and activity were at a lower level in infected leaves, sucrose accumulation was at a higher level in the same leaves.

Keywords: *Sugarcane mosaic virus*; sugarcane; RT-PCR; plant response

1. Introduction

Sugarcane or *Saccharum* spp., family Poaceae, is a widely cultivated crop that provides sugar across the globe. In Indonesia, sugarcane is widely cultivated on Java Island, particularly in East Java, and is the highest contributor to the national sugar production. During cultivation, this production is unstable due to several problems, including mosaic disease. Putra et al. [1] reported that sugarcane loss due to mosaic disease is about 20% with 50% of incidence.

In Indonesia, mosaic-like symptoms are present with various possible causative agents, including nutrient deficiency and plant viruses [1,2]. Typically, mosaic disease in the affected sugarcane shows yellowing and chlorosis on leaves, resulting in yield loss for both crop yield and sugar production [3]. On the other hand, mosaic symptoms caused by plant viruses such as *Sugarcane streak mosaic virus* (SCSMV), *Sugarcane mosaic virus* (SCMV), and *Sorghum mosaic virus* (SrMV) are difficult to distinguish by morphological observations of symptoms alone [4]. In addition, the typical symptom is an unstable source of information of the causative agent, because such symptoms are influenced by the time of infection, environment, and cultivar [2]. These viruses have been reported as dominant pathogens infecting sugarcane in several countries, including SrMV in China [5], SCMV and SrMV in Argentina [6] and the USA [7], and SCMV in Cuba [8]. Although several viruses may infect and show similar mosaic symptoms on sugarcane, it has been reported that the most widespread and dominant mosaic pathogens on sugarcane in Indonesia are SCSMV, SCMV, or both [1].

Thus, it is critical to accurately identify the causative agent of mosaic on sugarcane in East Java, Indonesia through biological, molecular, and serological assays [9], prior to deciding upon management and control strategies. Many reports on detecting the causative agent of mosaic on sugarcane have been conducted by a single or double methods such as RT-PCR [10] or a serological test [11]; however, each method presents its own disadvantages and advantages concerning accuracy and reliability.

A potyvirus, such as SCMV, is a single-stranded RNA virus with a simple genome structure encoding 10 mature proteins, specifically (from N-terminal to C-terminal) the first protein (P1), the helper component proteinase (HC-pro), the third protein (P3), the first 6K protein (6K1), the cylindrical inclusion protein (CI), the second 6K protein (6K2), the viral protein genome-linked (VPg), the nuclear inclusion a protein (NIa), the nuclear inclusion b protein (NIb), and the coat protein (CP) [12]. In addition, Moradi et al. [13] found that among genetic structure of SCMV, interspecific recombinants can be identified with two recombination patterns at the P1 coding region, depending on the host-plant of the virus. For example, SCMV from sugarcane (NRA) has recombination at six sites (at P1, HC-Pro, CI, NIa-Vpg, and NIa-pro coding regions), while SCMV from maize has four recombination sites (at P1, HC-Pro, NIa-Pro, and NIb coding regions). Interestingly, there is an Open Reading Frame (ORF) that overlaps P3, namely PIPO, expressing P3N-PIPO which is known to colocalize to plasmodesmata, where it acts in conjunction with CI to mediate cell-to-cell spread of the virus [14]. On the other hand, another frameshift protein may be present as overlapping ORF on P1 that expresses P1N-PISPO, which has so far only been identified in *Sweet potato feathery mottle virus* (SPFMV) [15].

During a virus infection, there are two possibilities of host-virus interaction that may occur as compatible or incompatible interactions. In the compatible interaction, the infection affects physiological, biochemical, and metabolic processes or changes in the plant, leading to symptom development due to systemic infection, activation, and suppression of global gene expressions in the host [16]. In the incompatible interaction, the virus infection triggers specific molecular interactions between the plant resistant (R) gene and viral avirulence (Avr) proteins, leading to the activation of a cascade of genes to induce defense mechanisms in the plant [17]. Several reports have demonstrated that various alterations in the plant as a response to virus infection have been indicated by some biochemical changes such as defense-related enzymes, carbohydrate accumulation, or photosynthetic and photo-assimilation activity [18]. Therefore, in this study, we performed experiments to identify the causative agent of mosaic disease on sugarcane in Indonesia through biological and molecular assays. In addition, plant response and alteration during infection were also studied morphologically and biochemically.

2. Materials and Methods

2.1. Sugarcane Leaf Samples, Disease Assessment, and Plant Inoculation

Sugarcane leaves, from both symptomatic (mosaic) and non-symptomatic plants, were collected from a Sugarcane Plantation company of Nusantara XI in East Java, Indonesia, and were assessed for disease incidence and severity. Briefly, disease incidence was assessed by calculating the number of symptomatic plants per total observed plants in the field, while disease severity was calculated by estimating the percentage of leaf area with mosaic symptoms using the following scoring system: 1 = no symptoms, 2 = 0.1%–2.5% leaf area showing symptoms, 3 =2.6–5%, 4 = 5.1–10%, 5 = 10.1–20%, 6 = 20.1–35%, 7 = 35.1–50%, 8 = 50.1–75%, 9 = 75.1–100% [15]. Samples were either directly processed for RNA isolation or stored at −80 °C to avoid the degradation of RNA by RNAse for identification purposes.

For the inoculation experiment, leaves from the symptomatic plant (cultivar NXI-1T) were homogenized with a mortar in 2 mL of phosphate buffer 0.1 M pH 8.0 (ratio 1:10) containing 2% of PVP (Polyvinylpyrrolidone). Plant sap was filtered and inoculated directly onto leaves of 6-week-old sugarcane PS 881 cultivar (seeds were obtained through tissue culture treated with 40 ppm of ribavirin, and were confirmed to be healthy through RT-PCR) with carborundum as an abrasive. Inoculated leaves were then rinsed with ddH_2O water to remove unnecessary material before incubation in a dark room overnight, prior to incubation in greenhouse. Mosaic development was observed on 10 replicate plants.

2.2. Total Plant RNA Extraction and Reverse Transcriptase Polymerase Chain Reaction

Frozen leaf samples (200 mg) were placed in liquid nitrogen and ground in a mortar. Total RNA was extracted using RNAeasy Mini Kit (Qiagen, Venlo, The Netherlands). The contaminant DNA was eliminated by DNAse (Merck KGaA, Darmstadt, Germany) treatment for 2 h. The quality of total RNA was checked in denaturing agarose gel electrophoresis and the quantity was determined using NanoVue Plus—UV Spectrophotometer.

First strand cDNA was synthesized from purified RNA. The mixture; 2 µg of purified RNA, 200U of M-MLV reverse transcriptase, 50 pmol of antisense primer (dT) and 1 mM dNTPs, was incubated at 42 °C for 1 h. The mixture was then heated at 70 °C for 10 min to stop the reaction. The cDNA was then PCR amplified using the synthesized primers (Bioneer, Daejeon, South Korea). The PCR reaction mixture contained 25 µL of 2×PCR Master mix Solution (i-Taq, iNtRON Biotechnology, Kyungki-Do, South Korea), 2 µL (100 ng) of template cDNA, and 1.5 µL of 10 pmol of pair primer. Primers used in this experiment were designated to amplify the coat protein sequence of SCMV using forward primer SCMV-F: 5′-TTT TCA CCA AGC TGG AA-3′ and reverse primer SCMV-R: 5′-AGC TGT GTG TCT CTC TGT ATT CTC-3′ [19], while for SCSMV using forward SCSMV-CPF2 5′-TCA TMT CTT CAT CRG CCG C-3′ and reverse primer SCSMV-CPR2 5′-ATC TTC YCT ACG CAG GTC CG-3′ [20]. PCR was performed by pre-denaturing at 94 °C for 2 min, followed by 40 cycles at 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 1 min, and lastly one cycle of final extension at 72 °C for 10 min. The 10 µL of PCR amplified product was analyzed by electrophoresis on 1% agarose gel.

2.3. Estimation of Total Chlorophyll, Phenol, and Peroxidase Activity

Total chlorophyll was estimated by following the procedure of Molazem et al. [21]. Two hundred and ten milligrams (210 mg) of finely cut fresh leaves were ground with 2.1 mL of 80% acetone. This mixture was then centrifuged at 3000 rpm for 10 min. The supernatant was carefully transferred and the procedure was repeated till the residue became colourless. The absorbance of the solution was read at 645 nm and 663 nm against the solvent (acetone) blank in 1 mL of supernatant using a spectrophotometer (UV-VIS double Beam, Hitachi, Japan). The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll were calculated using the following equation: Chlorophyll a

was calculated as $(12.7(A_{663}) - 2.69(A_{645})) \times 0.5$, while Chlorophyll b was calculated as $22.9(A_{645}) - 4.69(A_{663})$.

The total phenolic content in the leaf was estimated using the Folin–Ciocalteau method with slight modification [22]. Briefly, extracts (200 µL), 50% of Folin–Ciocalteau's reagent (100 µL), and distilled water (750 µL) were mixed and incubated in a tube for 3 min, and then 2% of Na_2CO_3 (300 µL) was added to the solution. The reaction mixture was mixed and incubated at 28 °C for 10 min. The mixture was then heated at 45 °C for 20 min prior to determining its absorbance at 755 nm. The sample was tested three times and a calibration curve with seven data points for gallic acid was established. The results were compared to a gallic acid calibration curve and the total phenolic content in the extraction of sugarcane was expressed as mg of gallic acid equivalents per gram of extracts per total protein.

Peroxidase activity was spectroscopically evaluated by measuring the absorbance of the reaction at 420 nm every 20 s for 2 min. Briefly, leaf extracts (5 µL) and 0.05 M of pyrogallol (150 µL) were mixed in a microplate, and then 1% of H_2O_2 (25 µL) was added and mixed before reading the absorbance using a spectrophotometer. All evaluations were performed in triplicate.

2.4. Analysis of Sucrose Phosphate Synthase, Rubisco, and Sucrose Accumulation in Leaves

Sucrose phosphate synthase (SPS) and rubisco were determined through Western blot analysis. Sodium dedocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed with equal amounts of leaf extracts (15 µg/mL of total protein content). Proteins were denatured and electrophoretically transferred to nitrocellulose membrane at 4 °C for 2 h. The membrane was then washed three times with Tris Buffer Saline (TBS). The SPS and rubisco protein abundance were evaluated by the detection of SPS and rubisco using specific polyclonal antibodies, and were visualized using chromogenic dye in conjunction between 25 µL of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and 50 µL of nitro blue tetrazolium (NBT) for every 10 mL of alkaline phosphate buffer.

Quantitatively, SPS activity was estimated by following Sawitri et al. [23]. Leaf extract was cleaned up using Sephadex G-25 and subjected to an enzyme activity assay. Twenty-five microliters (25 µL) of crude enzyme was mixed with 20 µL of buffer (composed by 86 mM MOPS-NaOH (pH 7.5), 26 mM $MgCl_2$, and H_2O), 10 µL of substrate (70 mM *fructose-6-phosphate*), 10 µL of 70 mM *uridine diphosphate glucose*, and 5 µL of 70 mM *glucose 6-phosphate* as the activator. The reaction was stopped by adding 35 µL of 1 M NaOH at particular times and incubated at 100 °C for 10 min. One portion of reagent (composed by 125 µL of 0.1% resorcinol and 375 µL of 30% HCl) was then added to the mixture and incubated at 80 °C for 8 min before measuring the absorbance at 520 nm.

Sucrose from the leaf extract was quantified by following Seliwanoff's method. Seventy microliters (70 µL) of 1 M NaOH was homogenized with 15 µL of leaf extract and heated at 100 °C for 10 min. After cooling, the solution was mixed with 250 µL of 0.1% resorcinol (in 95% of ethanol) and 750 µL of 30% HCl following incubation at 80 °C for 8 min, prior to determining the absorbance using a spectrophotometer (UV-VIS double Beam, Hitachi, Japan) at 520 nm. Each sample was analyzed in triplicate against the concentration of sucrose as a standard curve.

3. Results

3.1. Mosaic Disease Incidence, Severity, Symptom Development, and Its Pathogen

We studied five sugarcane cultivars from three different regions of sugarcane farm in East Java, Indonesia, including NXI 1T, VMC 7616, COKRO, PS 881, and PS 864. All cultivars were showing mosaic symptoms on leaves with different incidence and severity. Our data indicated that COKRO was the most resistant cultivar with 26% and 16.9% of disease incidence and severity, respectively, while NXI 1T and PS 881 were the most susceptible cultivars with about 78% and 63% of disease incidence, and 53% and 60.13% of disease severity due to the causative agent of mosaic disease, respectively, for both cultivars (Figure 1a).

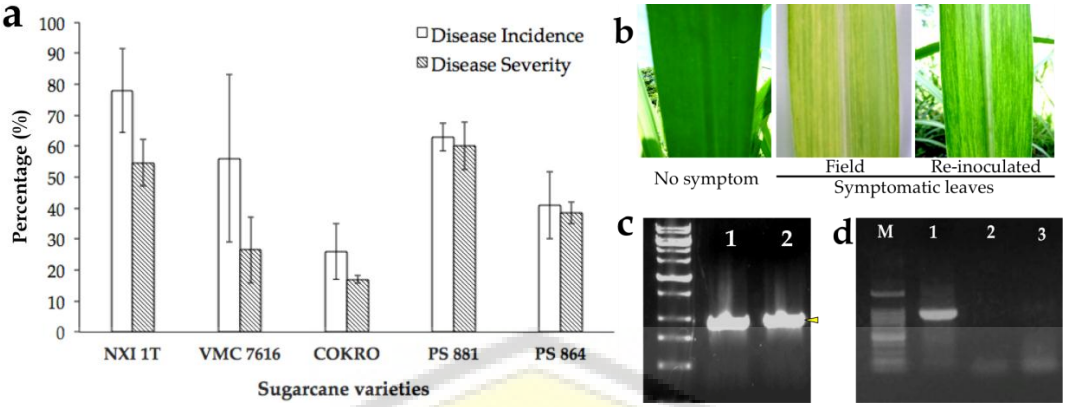

Figure 1. Disease incidence and severity were estimated on several cultivars of sugarcane in the field (**a**). Some leaves showed mosaic symptoms (**b**, middle and left). RT-PCR result of naturally occurring symptomatic plant (lane 1), and from re-inoculated sugarcane leaf (lane 2) of cultivar PS 881 using specific primer for *Sugarcane mosaic virus* (SCMV) (**c**), and RT-PCR result of symptomatic NXI-1T leaves (used as sap) using SCMV primer (lane 1) and *Sugarcane streak mosaic virus* (SCSMV) primer (lane 2) and on non-inoculated PS 881 leaves (lane 3) (**d**) used in this experiment.

To confirm the possible causative agent of mosaic disease, we re-inoculated sugarcane plants with plant sap of symptomatic plants (from the field) and observed the possible transmission of the symptom. All plants showed similar symptoms; the field symptomatic plants showed mosaic and yellowing along the sugarcane leaves (Figure 1b). Since there are some plant viruses which are able to infect sugarcane (such as SCMV, SCSMV, or SrMV) with the ability to induce very similar mosaic symptoms, we conducted RT-PCR analyses to diagnose the possible causative virus. The data confirmed that all symptomatic plants (both from the field and re-inoculated plants) produced a specific size of band. All samples (symptomatic plants and re-inoculated plants) showed a particular band at about 900 bp (Figure 1c). In addition, to confirm that the plant sap contained only one virus, we then detected the possible presence of widely distributed viruses in sugarcane using either SCMV or SCSMV pair primers. Our results proved that the plant sap used in the experiment contained only one virus, which was successfully amplified while using SCMV primer and not SCSMV primer (Figure 1d). This result indicates that the plant sap used in this experiment contained only SCMV as the representative for this study.

In addition, the observation of symptom development of inoculated plants showed that the first mosaic symptom appeared at 24 days post-inoculation (dpi) on the fourth leaf above the inoculation site (Figure 2a), and became clearer at the fifth leaf (Figure 2b). This observation indicated that infectious agents such as the virus cause the mosaic on sugarcane.

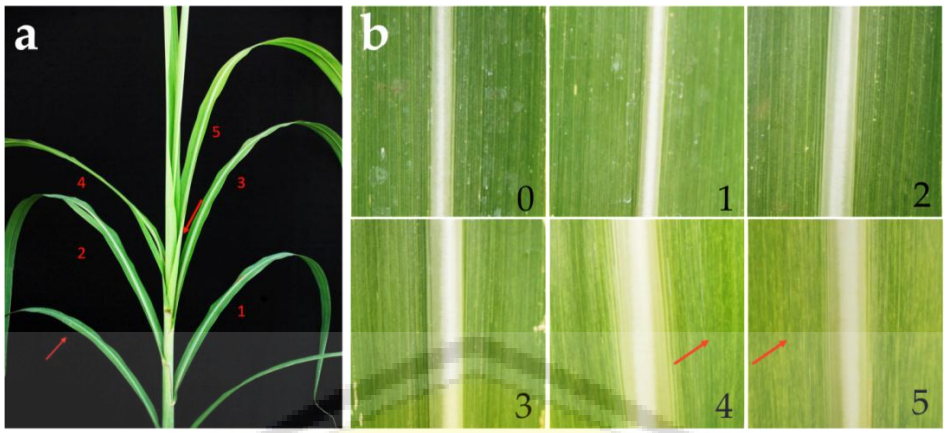

Figure 2. Sugarcane plant used in the inoculation experiment. (**a**) Plant was inoculated with symptomatic plant sap at the site of inoculation, indicated by number zero (0). (**b**) Representative sugarcane leaves from inoculated plant. Numbers indicate leaves upward from the inoculation site (1–5). Arrows indicate mosaic symptoms.

3.2. *Sugarcane Response and Its Alteration During Infection by SCMV*

During infection, we observed some properties of sugarcane such as total chlorophyll, peroxidase activity, and total phenol in leaves. Our results showed that total chlorophyll was drastically reduced (Figure 3a) in inoculated leaves, indicating that SCMV infection may alter or inhibit chlorophyll formation, while peroxidase activity and total phenol content had not significantly increased in inoculated leaves (Figure 3b,c).

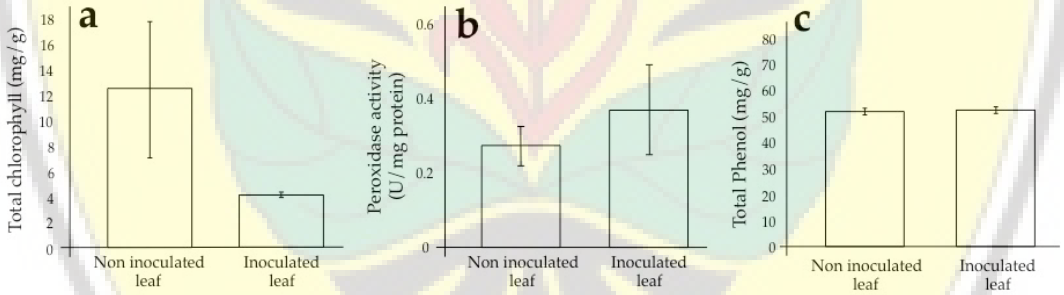

Figure 3. Alteration of biochemical properties in leaves of tested plants (PS 881 cultivar). (**a**) Total chlorophyll, (**b**) activity of peroxidase, and (**c**) total phenol accumulation. All data are estimated triplicates.

Interestingly, the results showed that SPS activity was in contrary to the sucrose content in the leaves. SPS activity was drastically reduced in inoculated leaves by about 50% (Figure 4a), while the sucrose content significantly increased in inoculated leaf by about 35% (Figure 4b).

To understand the possible reason for a reduction in SPS activity, we analyzed the SPS content in sugarcane leaves. Western blot analysis indicated that SPS was produced abnormally in inoculated leaves, but not rubisco. The abnormality of production of SPS was indicated by a smaller SPS signal detected using SPS polyclonal antibody (Figure 4c), while the internal control (rubisco, both large sub-unit (LSU) and small sub-unit (SSU)) showed a comparable amount in both non-inoculated and inoculated leaves (Figure 4c).

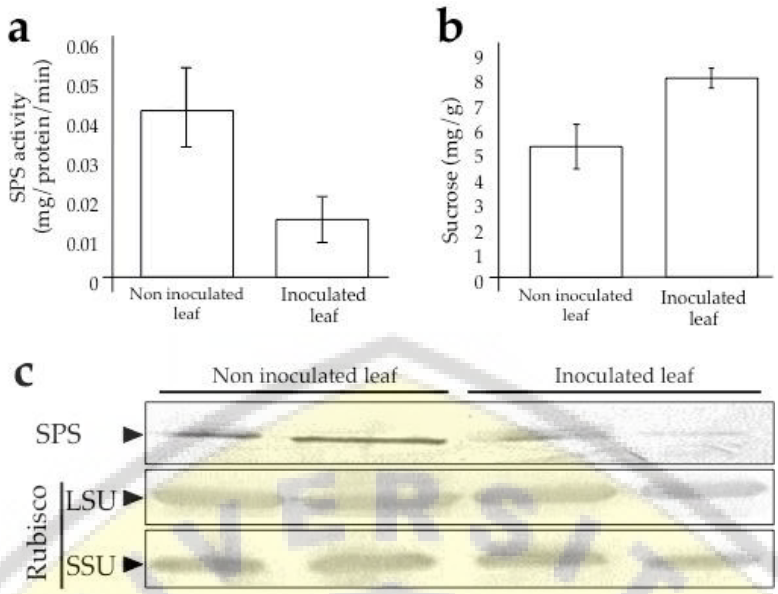

Figure 4. *Sucrose phosphate synthase* (SPS) activity assay (**a**), sucrose content (**b**), and Western blot on SPS and rubisco protein (**c**) from non-inoculated and inoculated sugarcane leaves. All data are estimated triplicates.

4. Discussion

Mosaic on sugarcane or on related plants might be caused by abiotic or biotic factors. One of the most common causes of mosaic on sugarcane is virus infection, specifically a potyvirus group such as *Sorghum mosaic virus* (SrMV), *Maize dwarf mosaic virus* (MDMV), or *Johnsongrass mosaic virus* (JGMV), including SCMV [24]. Infection of SCMV presents as irregular, light-green mosaic or a yellowish or chlorotic effect along the veins, and causes yield loss on several susceptible plants [25]. With a diagnosis based on the symptom, it is difficult to identify a particular causative virus because of the pattern similarity of symptoms. Many researchers have used several tools to detect these pathogens by examining virus particles using electron microscopy [26], enzyme-linked immunosorbant assay (ELISA) [11], or by reverse transcriptase polymerase chain reaction (RT-PCR) [20] combined with DNA sequencing, particularly on the coat protein gene fragment. The sequence of the coat protein gene for SCMV is commonly used to identifying mosaic pathogens, for instance the identification of mosaic disease on sugarcane and maize in Thailand [27], maize and canna in China [28–31], and sugarcane in Argentina [32]. Moreover, according to the coat protein sequence, the virus is also easily grouped into strain, because the sequence has unique parts among strains of SCMV related to their hosts [28], and more specifically, it has unique parts at the N-terminal amino acid residue of coat protein which is the second trypsin cleavage site and the residues which contain repeat sequence motifs [27]. In this research, we amplified the 900 bp cDNA fragment (Figure 1c) and suggested that the causative agent of mosaic in sugarcane was *Sugarcane mosaic virus*. WE employed a specific pair primer, as described in the methods section, which has been widely used to detect *Sugarcane mosaic virus* that infects plants. A similar pair-primer has also been used following confirmation by sequence analysis, which revealed that a particular band amplified by using the primer was responsible for the coat protein of SCMV [8,28–30]. Our suggestion that SCMV is the causative agent on inoculated sugarcane was proven by the diagnostic test of another possible virus like *Sugarcane streak mosaic virus* (SCSMV), which was negative as shown in Figure 1d. We did not detect the existence of *Sorghum mosaic virus* (SrMV), since that virus has not been found to present in Indonesia or at the plantation that performed monoculture practices. In addition, our results showed that mosaic development depended on sugarcane cultivars (Figure 1a), indicating that plant response might influence symptom development. Infection of SCMV may incite different responses from different cultivars, host species,

or stages of plant growth during infection, resulting in variation of symptom appearance or incubation time. Incubation of SCMV on maize, sorghum, and sugarcane varied about 4–15 dpi and was longer when transmitted through the seed (about 25–30 dpi, as reported by Gemechu et al.) [27].

SCMV is a plant pathogenic virus that systemically transmits and presents mosaic on younger leaves [32]. Our results showed that the mosaic appeared at the fourth leaf and younger leaves above the inoculation site (Figure 2b) and showed particular mosaic symptoms such as yellowing and chlorotic effects on leaves. This phenomenon indicates that virus infection develops in the plant systemically. During infection, the virus replicates and transmits into upper or younger leaves but requires and interval to produce mosaic symptoms. Our data showed that mosaic due to SCMV infection exhibited for the first time at the fourth leaf and became contrasted at the fifth leaf above the inoculated leaf (Figure 2a,b). Moreover, we also confirmed that mosaic on leaves is related to the alteration of chlorophyll content in infected leaves (Figure 3a). This suggests that mosaic on leaves indicated by yellowing and chlorotic effects reflect the altered pigmentation and structural change of chloroplasts, leading to depleted photosynthetic activity. Moreover, virus infection related to chloroplast is responsible for some changes such as chlorophyll pigmentation, photosystem efficiency, or photo-assimilate accumulation [33].

Peroxidase is an enzyme in plants that occurs in response to some stimuli such as pathogen infection, chemical agents, or mechanical agents [34]. In addition, some researchers have suggested that an increase in peroxidase activity is correlated with the degree of defense of the host plant, which involves lignin biosynthesis and cell wall reinforcement [35]. We were not able to draw conclusions about the phenomenon of the level of peroxidase activity and total phenol, content since they were observed to be at the same levels as the control plants, although mosaic symptoms were still observed on leaves (Figures 2b and 3b,c). However, we suggest that this condition might occur since the increased level of peroxidase activity was not at a significant level compared to the control plants (Figure 3b), indicating that the resistance response in sugarcane was low. This was supported by our data that the plant cultivar which we used in this study was the most susceptible cultivar (Figure 1a). Similarly, Bhargava et al. [36], observed that peroxidase activity increased in SCMV-infected sugarcane, indicating that infection affects the sugarcane physiology by inducing activity of catalase, in turn resulting in the higher activity of peroxidase to produce hydrogen peroxide. We suggest that although the plants exhibited a response against SCMV infection, they were unable to inhibit the development of SCMV, resulting in the appearance of symptoms. During the infection stage, the virus may change post-transcriptional gene silencing, alter particles movement, and affect host biochemical and physiological changes [37].

Interestingly, we observed an unusual phenomenon between SPS activity and sucrose accumulation in leaves (Figure 4a,b). We suggest that the lower activity of SPS in infected leaves occurred as a result of the inhibition of the plant to produce normal levels of SPS protein (Figure 4c). Less abundant SPS production caused lower SPS activity in leaves. However, the mechanism of how SCMV infection affects SPS protein biosynthesis remains unclear. Since SPS plays a crucial role in sucrose biosynthesis, incorporating with Sucrose Phosphate Phosphatase (SPP) [38,39], the increased activity of SPS would result in a higher sucrose accumulation [40]. In contrast, our result showed a lower SPS activity, but higher sucrose accumulation in infected leaves (Figure 4b). We suggest that this condition may occur during virus infection, resulting in the reduction of total chlorophyll (Figure 3b), which consequently leads to lower light absorption and abnormal phloem functionality [17]. On the other hand, the lower activity of SPS may be due to the higher sucrose accumulation itself by downregulating SPS by inhibiting the enzyme activity, but not its expression. This suggestion was supported by Salerno and Pontis [41] and Reimholzt et al. [42], in that sucrose reduced SPS activity by inhibiting and inactivating the enzyme.

Sucrose is the main photo-assimilate translocated from source to sinks via phloem. On the other hand, plant viruses remain in symplast [43] and need to move systemically via plasmodesmata (for cell to cell) or via phloem (for long distance), by which a virus-encoded protein facilitates its

movements and alters the size of plasmodesmata, leading to the impairment of photo-assimilate trafficking, including sucrose [44]. In addition, the modification or alteration of phloem in infected leaves affects the translocation of sucrose from source to sink, as previously reported by Shalitin and Wolf [14] on potyvirus infection in melon by *Cucumber mosaic virus* (CMV), which affects sucrose delivery resulting in the accumulation of sucrose in leaves that may downregulate SPS activity.

5. Conclusion

This study confirmed that *Sugarcane mosaic virus* (SCMV) was the causative agent of mosaic on sugarcane observed in East Java, Indonesia. Symptom of mosaic appeared on the fourth leaves upward from the inoculation leaf, in addition to showing some changes in that leaves including peroxidase, chlorophyll, as well as *sucrose phosphate synthase* (SPS).

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