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Recombinant of *Sugarcane streak mosaic virus* Coat Protein Polyclonal Antiserum Production and Its Serological Assays

Produksi Poliklonal Antiserum Rekombinan Protein Selubung *Sugarcane streak mosaic virus* dan Uji Serologinya

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ABSTRACT

Sugarcane streak mosaic virus (SCSMV) is one of the important viruses that causes streak mosaic disease in sugarcane plants. This virus spreads rapidly mechanically and is transmitted by cutting canes. Until now, a commercial SCSMV antiserum has not been available, even though it is essential for routine field detection and monitoring of disease development. A recombinant clone of the SCSMV coat protein gene (SCSMV-CP) in an expression vector has previously been successfully synthesized. This study aimed to produce a polyclonal antiserum by overexpressing recombinant SCSMV-CP in the *Escherichia coli* strain BL21(DE3) as an antigen and to test the antiserum's sensitivity for detecting SCSMV serologically. The sensitivity and specificity of the recombinant antiserum for detecting SCSMV were determined using the agar gel precipitation test (AGPT), Indirect ELISA (I-ELISA), and dot blot immunobinding assay (DBIA) methods. Recombinant SCSMV-CP antiserum was successfully produced in New Zealand rabbits, but it failed to detect SCSMV using the AGPT method. The sensitivity of SCSMV antiserum in the I-ELISA method was best up to an antiserum dilution of 1:1000 (v/v), and the sensitivity of the DBIA method was higher up to an antiserum dilution of 1:10 000 (v/v). SCSMV antiserum reacted negatively to SCMV antigen in the I-ELISA method, indicating the high specificity of the recombinant SCSMV-CP antiserum. This recombinant antiserum is suitable for routine detection of SCSMV using both I-ELISA and DBIA methods and has the potential for future mass production. The DBIA detection method is suitable for large-scale samples, is easy, fast, and accurate, and is cheaper than I-ELISA.

Keywords: antiserum, protein, recombinant CP gene; streak mosaic, sugarcane

ABSTRAK

Sugarcane streak mosaic virus (SCSMV) merupakan salah satu virus penting penyebab penyakit mosaik bergaris pada tanaman tebu. Virus ini menyebar dengan cepat secara mekanis, dan ditularkan melalui bagal/stek. Sampai saat ini antiserum komersial SCSMV belum tersedia, padahal sangat diperlukan untuk deteksi rutin dan pemantauan perkembangan penyakit di lapangan. Klon rekombinan gen selubung protein SCSMV (SCSMV-CP) dalam vektor ekspresi telah berhasil disintesis sebelumnya. Penelitian ini bertujuan memproduksi antiserum poliklonal menggunakan *overexpression recombinant* SCSMV-CP sebagai antigen pada *Escherichia coli* strain BL21(DE3) dan menguji sensitivitas antiserum untuk mendeteksi SCSMV secara serologi. Sensitivitas dan spesifisitas antiserum rekombinan untuk mendeteksi SCSMV diuji dengan metode *agar gel precipitation test* (AGPT), *Indirect* ELISA (I-ELISA), dan *dot blot immunobinding assay* (DBIA). Rekombinan SCSMV-CP antiserum berhasil diproduksi pada kelinci New Zealand, namun tidak dapat mendeteksi SCSMV dengan metode AGPT. Sensitivitas antiserum SCSMV pada metode I-ELISA terbaik sampai pengenceran antiserum 1:1000 (v/v), dan sensitivitas metode DBIA lebih tinggi sampai pengenceran antiserum 1:10 000 (v/v). Antiserum SCSMV bereaksi negatif terhadap antigen SCMV pada metode I-ELISA, mengindikasikan spesifisitas rekombinan SCSMV-CP antiserum yang tinggi. Rekombinan antiserum ini cocok untuk deteksi rutin SCSMV baik dengan metode I-ELISA maupun DBIA dan berpotensi untuk diproduksi massal di masa datang. Metode deteksi DBIA sesuai digunakan untuk mendeteksi banyak sampel, mudah, cepat, akurat dan lebih murah dibandingkan I-ELISA.

Kata kunci: antiserum, gen CP rekombinan, mosaik bergaris, protein, tebu

INTRODUCTION

Sugarcane streak mosaic virus (SCSMV-Potyviridae: *Poacevirus*) is an important virus that infects sugarcane plants (Dougrois *et al*. 2024). Its occurrence in Indonesia was first reported in Java (Damayanti and Putra 2011), and subsequently became widespread in Lampung and South Sulawesi (Subekti *et al*. 2020a). Nowadays, SCSMV distributed in numerous Asian countries, Western hemisphere and Africa (Daugrois *et al*. 2020; Sorho *et al*. 2021; Dougrois *et al*. 2024). The specific symptoms of SCSMV include streak mosaics, and severe infections can lead to pale leaf color, yellow mosaic lines on the leaves, and a more transparent appearance compared to healthy leaves (Prabowo *et al*. 2014). A disease incidence of 50% can cause a decrease in sugar production of 16%–17% to 19%–21% (Asnawi 2009). The significant decline in sugar production due to SCSMV infection is one of the obstacles to the success of the national sugar self-sufficiency program in Indonesia.

SCSMV can be transmitted mechanically and vegetatively through infected cutting cane. Virus transmission through vegetative sugarcane material is important because sugarcane propagation primarily occurs through vegetative cutting. Transmission through cutting knives during propagative cutting cane preparation before planting or at harvest time, can efficiently spread SCSMV, and the distribution of planting materials from one area to another can occur regardless of the health of the cane (Damayanti and Putra 2011; Putra *et al*. 2015).

Generally, utilizing resistant varieties/clones against viruses transmitted via propagative material, such as sugarcane, is the most effective strategy for controlling virus diseases (Putra *et al*. 2023). Among the 27 varieties reported, six were highly resistant to mechanical infection by SCSMV. However, their resistance needs to be evaluated on a field scale (Ernawati *et al*. 2022). Additionally, providing virus-free cutting cane is a crucial step in preventing infection and transmission in the field as an initial preventive measure. Therefore, an easy and highly accurate detection system is needed for routine detection in providing healthy sugarcane cutting cane.

Serological assay is a common method for detecting plant viruses, especially because it can handle large sample sizes with a sufficient level of accuracy. Serological assays that can be used to detect viruses include mass spectrometry analysis, double-diffusion test, dot blot immunobinding assay (DBIA), tissue-blot immunobinding assay (TBIA), western blotting, agarose gel precipitation test (AGPT), and enzyme-linked immunosorbent assay (ELISA) (Kirankumar *et al*. 2020). ELISA, DBIA and TBIA are modern and most popular serological methods to detect viruses in large number of plant samples (Abd El-Aziz 2019). The success and accuracy of these serological assays are highly dependent on the availability of good-quality antiserum (Kumari *et al*. 2006). Unfortunately, the SCSMV commercial antiserum is not available yet.

Advances in molecular biology technology have enabled the production of antigen molecules by expressing specific genes inserted into an expression vector plasmid in *Escherichia coli*. The advantages of antigen preparation using this method include the availability of sufficient antigen at all times when needed for antiserum production (Cotillon *et al*. 2005). The method is also suitable for preparing antigens from viruses with low concentrations in plant tissue, viruses that infect hard plant tissue, and viruses with limited distribution within plant tissue (phloem-limited) (Aseel and Hafeez 2017). This method of antigen preparation for antiserum production has been successfully reported for several viruses, including SCSMV (Hema *et al*. 2003a), *Blackeye cowpea mosaic virus* (Koohapitagtam and Nualsri 2013), *Potato virus* X, *Potato leafroll virus* (Abdel-Salam *et al*. 2013), *Tomato infectious chlorosis virus* (Kurniawati *et al*. 2015), *Sugarcane mosaic virus* (SCMV) (Darsono *et al*. 2018; Astuti *et al.* 2019), *Sweet potato latent virus*-Lotus (He *et al*. 2020), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) (Paradisa *et al.* 2022), and *Soybean mosaic virus* (SMV) (Ren *et al*. 2022).

Given the increasingly widespread distribution of SCSMV in Indonesia and its devastating impact on national sugar production, a strategy is needed to suppress infection and spread of this disease, i.e. by conducting routine disease monitoring and detection especially on crops in the field. Serological methods are one option for SCSMV detection. Creating a high-quality antiserum will enable better virus detection, support field monitoring, and help ensure virus-free planting material. In a previous study, we constructed and successfully expressed the SCSMV-CP gene in *E. coli* strain BL21(DE3) (Hamdayanty *et al*. 2016). In this paper we reported the production a polyclonal antiserum against recombinant SCSMV-CP and test it to detect SCSMV serologically.

MATERIALS AND METHODS

Antibody Production and Purification

The SCSMV isolate from Pasuruan Regency, East Java was obtained from the sugarcane PS864 clone and it showed typical symptoms. The DNA sequence of this isolate was deposited in the GenBank database with accession number AB563503 (Damayanti and Putra 2011; Hamdayanty *et al*. 2016).

Antiserum was produced in New Zealand white female rabbits. Two female New Zealand white rabbits were immunized subcutaneously with 500 µg

of purified recombinant SCSMV-CP fusion protein. It was emulsified with 500 µL of Freund's complete adjuvant at a ratio 1:1 (v/v). Three weeks after the first immunization, the rabbits were boosted with two additional subcutaneous injections of 150 µg of the purified protein mixed with 150 µL of Freund's incomplete adjuvant, administered four times with three-week intervals. The fourth injection was done without using adjuvants and injected into the auricular veins in the rabbit's ears. Blood samples were collected every 2 weeks after immunization to assess the antiserum titer, up to 1.5–2 mL via the auricular vein using a 3 mL disposable syringe.

Blood samples were collected a week after the fourth immunization, when the SCSMV antiserum titer was high in the blood. Blood samples were allowed to coagulate for 1 hour at room temperature and then centrifuged at 2000 rpm (±450 × g) for 10 min. The antisera were aliquoted and stored at -20 °C in a freezer.

The produced serum was purified by precipitation with saturated ammonium sulfate as described by Assaat *et al*. (2018). Ammonium sulfate was added to the serum with a final concentration of 40%, incubated for 18 hours at 4 °C, and centrifuged at 3800 rpm (±1600 × g) for 30 minutes. The precipitate was resuspended in 0.01 M phosphate-buffered saline (PBS) to 0.4× the initial serum volume. The serum mixture was dialyzed in PBS 0.01 M, pH 7.0 (1:100 v/v) at 4 °C with PBS replacements every 6 hours. The protein concentration in serum, which reflects the antiserum concentration, was determined by measuring absorbance at 280 nm.

Serological Assay

The antiserum produced was tested using 3 methods, i.e. agarose gel precipitation test, indirect enzyme-linked immunosorbent assay, and dot blot immunobinding assay.

Agarose Gel Precipitation Test (AGPT). Antigen-antibody reactions to AGPT were carried out on 1% agarose gel (Sigma, US). The gel was made by dissolving 0.1 g agarose and 0.01 g sodium azide (NaN_3) in 5 mL PBS pH 7.2 and 5 mL distillated water, which were heated in the microwave for 1 minute. Liquid agarose is poured on a glass plate about 2 mm thick and left at room temperature until it solidifies.

After the agarose gel solidifies, holes are punched into it using a mold (puncher) with seven hexagonal holes. The antigen suspension was prepared by grinding 0.1 g of sugarcane leaves, which were positively attacked by SCSMV, in 1× PBS with a ratio of 1:10 (b/v). A pure antigen, in the form of the CP-SCSMV protein, was purified for comparison. Each antigen and antiserum was inserted into the hole in an agarose gel of 25 µL. The agarose gel was then incubated for 24–48 hours in a dark place at room temperature. The formation of precipitation lines of antigen-antibody reactions on agarose gel media was observed daily and documented by digital camera. The test was conducted ten times replication.

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA). In this method, dilutions of 1:100, 1:250, 1:500, 1:750, 1:1000, and 1:2000 (v/v) of the antiserum and of the diseased plant juice are also carried out in buffer at 1:10, 1:50, 1:100, 1:500, and 1:1000 (w/v). Sugarcane plant leaf samples as antigens (0.1 g) were crushed in a coating buffer [1.59 g Na_2CO_3, 0.293 g $NaHCO_3$, 0.20 g NaN_3, 20 g polyvinylpyrrolidone (PVP) in 1 L of sterile water, pH 9.6], then as much as 100 µL of juice (plant sap) was filled into ELISA wells and incubated overnight at 4 ºC. After that, the plates were washed 4–8 times with 1× phosphate-buffered saline Tween-20 (PBST) [8 g NaCl, 2 g KH_2PO_4, 1.15 g Na_2HPO_4, 0.2 g KCl, and 0.5 mL Tween-20 dissolved in 1 L sterile water, pH 7.4].

A total of 100 µL of recombinant CP-SCSMV antiserum was dissolved in conjugate buffer [2 g bovine serum albumin, 20 g PVP, 0.2 g NaN_3 in 1 L of sterile water, pH 7.4], incubated at 37 °C for 2 hours, and washed with 1× PBST as many as 4–6 times. Each well was then filled with 100 µL universal antiserum goat anti-rabbit-IgG alkaline phosphatase (GAR-AP) (1:1000 v/v), which had been dissolved in conjugate buffer and incubated at 37 °C for 2 hours. The ELISA well is then washed 4–6 times with 1× PBST. After that, each well was filled with 100 µL of the p-nitrophenyl phosphate (PNP) substrate. One tablet of PNP (5 mg) was dissolved in 5 mL PNP buffer [97 mL diethanolamine, 0.2 g NaN_3, 0.1 g $MgCl_2$ in 1 L sterile water, pH 9.8] and incubated at room temperature until the color changes to yellow.

The ELISA results are quantified using an ELISA reader at 405 nm (Thermo Fisher). The sample is positive if the ELISA absorbance value (EAV) of test sample is 2 times greater than the negative control (healthy plants) (Kashina *et al*. 2007). The test was repeated twice with duplicate samples for each antiserum dilution.

Dot Blot Immunobinding Assay (DBIA). The DBIA was conducted according to the procedure used by Lu *et al*. (2026). Healthy sugarcane leaf, sugarcane leaf infected by SCSMV, and SCMV each were crushed using 1× tris buffer saline (TBS) (0.02 M Tris HCl, 0.15 M NaCl, pH 7.5) in a ratio of 1:10 (w/v). A 2 µL of antigen was blotted on the nitrocellulose membrane and incubated at room temperature until the juice is perfectly absorbed on the membrane.

The membrane was blocked with a 1× TBS solution containing 2% skim milk and 2% Triton X-100,

incubated in a shaker at 150 rpm for 2 hours. Then, the membrane was washed by soaking in distilled water and incubated for 5 minutes for 5 times. The membrane was then immersed in the SCSMV recombinant antiserum dissolved in TBS buffer containing 2% skim milk and incubated for 2 hours. The antiserum was serially diluted, i.e. 1:100, 1:500, 1:1000, 1:2000, 1:3000, 1:4000, 1:5000, and 1:10 000 (v/v) to determine the sensitivity of the assay. After that, the membrane was washed with Tris-buffered saline Tween-20 (TBST) (TBS + 0.05% Tween-20) for 5 min, and the wash was repeated 5 times.

The membrane is then immersed in the antiserum goat anti-rabbit-IgG alkaline phosphatase (GAR-AP), which is dissolved in TBS + skim milk 2% ratio 1:5000 (v/v). The membrane was washed with TBST for 5 minutes, then repeated for 5 times. Furthermore, staining is performed by soaking the membrane in a nitro blue tetrazolium (NBT) substrate at 75 mg mL^{-1} and 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) at 50 mg mL^{-1}. The nitrocellulose membrane soaked in NBT/BCIP (66 µL of NBT and 30 µL BCIP) dissolved in 10 mL alkaline phosphate buffer (0.1 M Tris HCl, 0.1 M NaCl, 5 mM $MgCl_2$). The positive reaction of SCSMV is marked by a color change in the sample on the membrane to purple. The test was repeated three times.

RESULTS

The SCSMV recombinant antiserum was successfully produced using recombinant clone technology overexpressed in *E. coli* strain BL21(DE3). Serological assay using blood serum, either at the first or second antiserum collection, by AGPT method, did not show any precipitation lines (data not shown). It indicates that the AGPT method has a low sensitivity level. This method requires very high concentrations of antigens and antibodies to get positive results.

Serological assay of blood serum from the second and third blood collection using I-ELISA method showed positive results, which were indicated by ELISA absorbance value (EAV) of SCSMV samples twice as high as those of EAV negative controls (healthy sugarcane) (Table 1). However, the EAV of SCSMV samples remained very low after prolonged incubation of the colorimetric reaction with p-nitrophenyl phosphate; the EAV was no more than three folds that of negative controls, indicating a low concentration of SCSMV antiserum in blood serum.

Serum testing from the fourth blood sample collection showed a higher EAV, reaching 8 times that of negative controls. The total blood obtained from first and second rabbits was ±75 mL and ±70 mL, respectively, and the total serum obtained was ±35 mL and ±30 mL, respectively. Total blood harvest was immediately done to avoid a decrease in antiserum titers in rabbit blood. It was reported in the previous experiment that the antiserum from the first blood collection was more sensitive to detecting the *Pelargonium zonate spot* virus than the antiserum from the second to fourth blood sampling (Gulati-Sakhuja *et al*. 2009). Further, the antiserum titers of *Blackeye cowpea mosaic virus* from the second to seventh blood sampling increased, and decreased titers to 4× lower in the eighth blood sampling (Koohapitagtam and Nualsri 2013).

Serological assay with I-ELISA showed high sensitivity of the antiserum in detecting SCSMV, both in crude and purified forms. Antigens from leaves infected with SCSMV elicited positive reactions with antiserum dilutions of up to 1:1000 (Table 2). The optimal and efficient antigen dilution for SCSMV detection was 1:10 with a 1:750 (v/v) antiserum dilution. Antiserum dilution of 1:100 still showed relatively high ELISA absorbance values in negative controls. Therefore, antiserum dilution of 1:100 (v/v) was not recommended to detect SCSMV by I-ELISA method.

Detection of SCSMV by I-ELISA using crude antiserum in this study showed that the sensitivity was not much different from that of the purified antiserum.

Table 1 The ELISA absorbance values (EAV) results of I-ELISA using serum obtained from different blood collection times

Blood collection	Serum dilution	ELISA absorbance values	
		Negative control	SCSMV sample
2	1:100	0.088[a]	0.199 (+)
	1:500	NC[b]	0.117
3	1:100	0.161	0.346 (+)
	1:500	0.072	0.144 (+)
4	1:100	0.141	1.149 (+)
	1:500	0.120	0.612 (+)

[a]ELISA absorbance value (EAV) was calculated at 45 minutes after the addition of the coloring substrate. Positive results if the EAV SCSMV sample is 2× greater than the negative control. The EAV obtained from two replicates test, each test consisted of duplo samples
[b] NC, ELISA for negative controls with a dilution of 1:500 antiserum was not carried out.

The results showed that the SCSMV antiserum produced can effectively detect SCSMV well, even without purification. The SCSMV polyclonal antiserum also showed quite a good specificity, which was indicated by the absence of SCMV detection. This is indicated by the ELISA absorbance values of SCMV samples, which did not reach 2-fold the EAV of negative controls (Table 3). The specificity of SCSMV antiserum to SCMV is important because SCMV also causes mosaic symptoms in sugarcane plants that resemble those caused by SCSMV.

Serological assay using the DBIA method are more sensitive than I-ELISA detection. The purple color was still observed until the 1:10 000 dilution of antisera, indicates the high sensitivity of the DIBA method (Figure 1). However, in DBIA detection, dilutions of

Table 2 Sensitivity of SCSMV polyclonal recombinant antiserum in I-ELISA at various SCSMV antigen dilution rates

Antigen SCSMV dilution	Antiserum dilution*					
	1:100	1:250	1:500	1:750	1:1000	1:2000
			Crude antiserum			
Buffer	-	-	-	-	-	-
Negative control	-	-	-	-	-	-
1:10	+++	+++	++	+++	++	+
1:50	++	++	+	++	++	+
1:100	++	++	++	++	+	+
1:500	++	++	++	++	++	-
1:1000	+	+	+	+	+	-
			Purified antiserum			
Buffer	-	-	-	-	-	-
Negative control	-	-	-	-	-	-
1:10	++	+++	+++	+++	++	+
1:50	++	++	++	++	++	-
1:100	++	++	++	++	+	+
1:500	++	++	++	++	++	-
1:1000	+	++	++	++	+	-

*-, Negative reaction; +++, Strong reaction if the ELISA absorbance value (EAV) ≥ 6 times EAV negative control; ++, Moderate reaction if EAV 4 - 5.99 times EAV negative control; and +, Weak reaction if EAV 2 - 3.99 times EAV negative control.

Table 3 Specificity of SCSMV polyclonal antiserum against SCMV antigens

Dilution of antiserum	Buffer	ELISA absorbance value*		
		Negative control	SCSMV antigen 1:10	SCMV antigen 1:10
1:100	0.173	0.195 / -	0.726 / +	0.253 / -
1:250	0.148	0.172 / -	0.629 / +	0.236 / -
1:500	0.136	0.145 / -	0.484 / +	0.223 / -
1:750	0.137	0.144 / -	0.419 / +	0.215 / -
1:1000	0.131	0.138 / -	0.367 / +	0.206 / -
1:2000	0.135	0.134 / -	0.294 / +	0.188 / -

*The ELISA absorbance value (EAV) was calculated at 45 minutes after the addition of the PNP substrate. The test is positive (+) if the EAV sample is 2× EAV negative control (healthy plants).

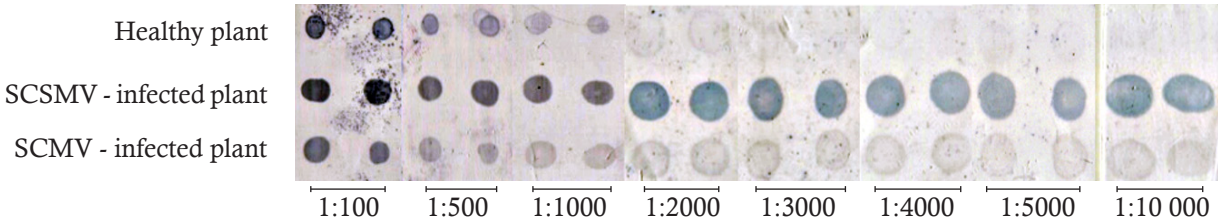

Figure 1 The sensitivity and specificity of SCSMV antiserum in the DBIA test by using antiserum dilutions of 1:100-1:10 000. SCMV antigen as a comparison to test the specificity of SCSMV antiserum.

antiserum from 1:100 to 1:1000 (v/v) indicate a purple color change in healthy plants (negative control). Color changes also occur in sugarcane leaf samples infected only with SCMV, even at a 1:1000 dilution of antiserum, with a very low purple intensity compared to the positive control sample for SCSMV. The optimum dilution of antiserum for detecting SCSMV with the DBIA method is $1:2000 \leq x \leq 1:10\,000$, which is indicated by the absence of purple discoloration in healthy plants and plants infected with SCMV.

DISCUSSION

Based on the results, the SCSMV recombinant antiserum was successfully produced. Serological methods possess advantages such as high sensitivity, accuracy, simple operation, and easy to observe visually (Boonham *et al*. 2014). Among the serological methods, the AGPT method was not suitable for the rapid detection of SCSMV from infected plants unless using antigens derived from purified SCSMV-CP recombinant protein. It indicates that to form precipitation lines, the method requires a high concentration of viruses. The absence of precipitation lines between SCSMV recombinant antiserum and antigens from leaf sap infected with SCSMV can also be caused by the mobility of flexuous filamentous SCSMV particles. Antigen diffusion in AGPT depends on concentration and is strongly affected by factors like particle size and shape. Smaller and compact particles diffuse more easily, while long or filamentous viruses move less in porous matrices. This reduced movement may prevent visible precipitation lines in the AGPT assay (Dechadilok and Deen 2006). Isometric and small viruses are very suitable for the AGPT method, whereas rigid-rod viruses will diffuse very slowly or not at all (Varma and Singh 2020).

The recombinant SCSMV antiserum exhibits high sensitivity and specificity, enabling confident differentiation between SCMV and SCSMV in I-ELISA (Table 3). Natural mixed infections of SCSMV and SCMV cause mosaic-like symptoms, complicating diagnosis. Therefore, having a reliable SCSMV recombinant antiserum that meets both sensitivity and specificity criteria is essential for accurate virus identification and effective disease management.

Based on the three serological tests using recombinant SCSMV antiserum, it was shown that DBIA had the highest sensitivity level. However, there was a chance of cross-reaction at low antiserum dilution (1:100 to 1:1000). Therefore, to overcome the cross reaction in healthy control, increasing the dilution of the antiserum while still obtaining a positive reaction with the positive control is important point in this method. The advantage of using detection with DBIA compared to ELISA is that the time needed is shorter, higher sensitivity levels, cost effective, fewer antiserum and chemicals needed (Varma and Singh 2020; Lu *et al*. 2026).

The recombinant SCSMV protein is capable of eliciting an immunogenic response in rabbits and exhibits the highest antibody titer after the fourth antigen injection. The produced SCSMV recombinant polyclonal antiserum was able to detect SCSMV in sugarcane using the I-ELISA and DBIA serological methods. The optimal comparison of antiserum for detecting SCSMV with I-ELISA is 1:750 (v/v) with a ratio of antigen 1:10 (w/v), and a ratio of antiserum to 1:10 000 (v/v) in DBIA detection is still able to detect SCSMV properly.

Although antisera available commercially, but it must be imported therefore to reduce the dependence import commercial antisera, attempt to produce antiserum specific virus by employing an advance biotechnology is worth further studies for various virus species in Indonesia. Some studies revealed that producing specific recombinant antiserum against specific viruses is necessary because its highly specificity over conventional produced antiserum. The advantage in producing recombinant antiserum in compare to conventional produced antiserum such as a large number of a single protein encoded by the virus, without low expression of non-structural protein and poor stability. The conventional antiserum prepared by using purified virus particles as antigens. However, it is many cases the effect of host proteins could not be rule out affect to low titer and poor specificity (He *et al*. 2020).

Previously, SCSMV recombinant antiserum was successfully produced using a similar approach and evaluated for sensitivity and specificity using IC-RT-PCR and I-ELISA (Neliana *et al*. 2025). The differences between the present study and the previous report are in the evaluation of the recombinant antiserum, focusing on serological tests for routine, and practical detection of a large number of samples. Among the tested serological methods, I-ELISA and DBIA are the best, with high sensitivity and specificity for routine detection. However, the DBIA methods is more practically suitable to detect or screening a large number of samples to identify the virus, especially in laboratories with limited facilities. DBIA was eight times more sensitive for detection of PVX and four times more sensitive for detection of PVS and PVY than DAS-ELISA (Abd El-Aziz 2019). Thus, it is necessary to develop a practical serological test for SCSMV that yields results comparable to those of I-ELISA.

Technical difficulties have hampered RT-PCR of SCSMV, making it difficult to obtain suitable RNA preparations from sugarcane tissues, and it is not recommended to large-scale indexing. However,

SCSMV RNA can be easily released from sugarcane leaves, stalks, and sheaths using ELISA washing buffer (phosphate-buffered saline Tween-20, PBST) in a single tube (simple direct tube, SDT) and can serve as a template for RT-PCR (Damayanti and Putra 2012). SDT-RT-PCR is a useful, convenient method for detecting viruses for which specific primers are available but for which antiserum is not yet available for routine detection (Suehiro *et al*. 2005). Further, qPCR has also been developed to detect SCSMV due to its high sensitivity and ability to quantify DNA in real time and rapidly (Subekti *et al*. 2020b). IC-RT-PCR is more sensitive than direct antigen-coating ELISA and DBIA; it is suitable for detecting SCSMV in breeding, germplasm, and quarantine programs (Hema *et al*. 2003b; Neliana *et al*. 2025).

Based on the results, the recombinant SCSMV-CP antigen expressed in *E. coli* BL21(DE3) proved suitable for producing a high-quality antiserum. SCSMV recombinant antiserum from this study can be used to detect SCSMV serologically. This recombinant antiserum provides a fast and reliable serological assay for SCSMV detection. Early detection of SCSMV, especially in sugarcane parental stocks, is expected to play a significant role in supplying virus-free cutting cane, thereby preventing its spread. Among serological test, the I-ELISA and DBIA methods are sensitive, easy to use, inexpensive, suitable for the detection of large numbers of samples, and suitable for a laboratory with minimal facilities. I-ELISA and DBIA can be utilized to obtain virus-free cutting canes prior to planting in routine detection activities, to monitor the development of SCSMV infection in commercial fields, and for epidemiological studies. In addition, the recombinant SCSMV-CP antiserum is promising for future mass production.

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