Immunocapture reverse transcription polymerase chain reaction for detection of sugarcane streak mosaic virus

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Abstract. Detection of plant viruses can be done by protein or nucleic acid approaches. The immunocapture-reverse transcription-polymerase chain reaction (IC-RT-PCR) method is a combination of the two approaches. Research was carried out to develop and validate IC-RT-PCR based-detection method for SCSMV, which can be applied for the sugarcane seed indexing program to support the national government's goal for sugar self-sufficiency. Evaluation of the IC-RT-PCR method was conducted using 5 field samples. Conventional PCR and serological methods, i.e. dot immunobinding assay (DIBA) and enzyme-linked immunosorbent assay (ELISA) was also performed in the same time. All field samples gave a positive reaction to SCSMV antibodies in the DIBA and ELISA methods with the intensity of the reaction varying from low to high. SCSMV was still detected on plant extract up to 10⁴ dilution by ELISA and DIBA. Specific DNA fragments were successfully amplified from 2 field samples using the conventional PCR method; whereas the IC-RT-PCR method was successfully amplified all field samples. Optimization test showed that the IC-RT-PCR method was able to detect SCSMV from plant extract up to 10⁶ dilutions. IC-RT-PCR method is more sensitive than conventional PCR and might be recommended for the indexing method to produce high-quality virus-free sugarcane seed.

Keywords: virus-free seed, dot immunobinding assay, enzyme-linked immunosorbent assay, IC-RT-PCR, seed indexing

1. Introduction

Sugarcane streak mosaic virus (SCSMV) is a major virus that infects sugarcane in Indonesia and other sugarcane producing countries. Detection and identification of SCSMV has been usually done by conventional polymerase chain reaction (PCR) methods. SCSMV specific primers, i.e. SCSMV AP3/547F have been developed previously and successfully detected the virus from infected plants [1, 2]. Although conventional PCR is a reliable method to detect SCSMV, this method has several limitations especially for samples with low virus concentration and those containing inhibitor compounds [3].

In addition to conventional PCR, Immunocapture (IC)-PCR has been developed as a combination of serological and molecular methods. The method has been developed for detection plant viruses like...
BSV, CsCMV, GLRaV-3 [4, 5, 6]. The IC-PCR method has been succeeded in developing by using recombinant antiserum for SCSMV with high sensitivity and specificity [2]. Sensitivity of RT-PCR was 62 times higher than ELISA to detect Tobamovirus and 100 times to PPV based on assessed several detection methods including IC-PCR and ELISA [7, 8]. In this article, we will discuss the results of IC-PCR method optimization for detecting SCSMV from infected sugarcane samples. Once the method is established, it can be applied for the sugarcane seed-indexing program to improve crop productivity.

2. Materials and methods

2.1. Time and location

The research was conducted from January to March 2019 at Plant Virology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB University and Biomolecular Laboratory, Center for Diagnostic Standards of Agricultural Quarantine, Ministry of Agriculture.

2.2. Sample preparation

Virus inoculum was obtained from Plant Virology Laboratory, IPB University and it has been identified previously as SCSMV-Pasuruan [1]. The virus is propagated in sugarcane clone PS 864, which is known as a susceptible clone. Infected sugarcane was maintained under greenhouse condition. Leaf extract was prepared in general extract buffer (GEB) (1:10 b/v); the stock leaf extract was then diluted serially in GEB with a dilution factor of 10^1 -10^10.

2.3. Immunocapture (IC) optimization

IC method was conducted following the Reddy’s protocol with modification in buffer solution and incubation period [5]. Anti-CP SCSMV was obtained from Biosecurity Laboratory, Sugar Research Australia. Antiserum was dissolved in coating buffer (1: 1,000 v/v), and then 50 µl of solution was placed into a microtube and incubated overnight at 4 ºC. After the incubation ends, the antiserum solution was removed and the microtube was washed with PBST twice and with PBS once. Leaf extract was then placed into the microtube, i.e. 50 ml per tube and incubated overnight at 4 ºC. As the last stage, the tube was washed in the same way as described previously. The tube is now determined to contain antisera binding-SCSMV and will be used for cDNA synthesis.

2.4. cDNA synthesis

cDNA synthesis was performed by reverse transcription method. Each reaction mixture consisted of 2 µl RT buffer (5x), 0.5 mM dNTP (Thermo Scientific), 5 mM µl DTT (Thermo Scientific), 0.35 µl RNase inhibitor (Thermo Scientific) (40 units/µl), 0.35 µl M-MuLV (Thermo Scientific) (200 units/µl), 5.7 µl nuclease-free water, and 0.75 µM oligo d(T) (Thermo Scientific). All of the reagents were added into the antisera binding-SCSMV tube. Synthesis of cDNA followed the reverse transcription reaction, i.e. incubation at 65 ºC for 5 min, 37 ºC for 60 min, and 70 ºC for 5 min.

2.5. cDNA amplification

Amplification of cDNA sample was performed in Applied Biosystem Veriti® 96 Well Thermal Cycler, Thermo Fisher Scientific, USA. Each reaction mixture consisted of 0.2 µM SCSMV AP3 primer, 0.2 µM primer SCSMV 547F, 2.5 µl of PCR buffer (10X), 0.875 mM MgCl2, 0.5 mM dNTP, 0.2 µl Taq polymerase, 16.225 µl ddH2O, and 1 µl cDNA sample. Amplification program started with a pre-denaturation of 94 ºC for 5 min; followed by 35 cycles which consisted of denaturation at 94 ºC for 30 sec, annealing at 51 ºC for 1 min and extension at 72 ºC for 2 min; then ended with heating at a temperature of 72 ºC for 10 min and cooling at 4 ºC. Visualization of DNA amplicons was performed on 1.2 % agarose gel in 1x TAE at a voltage of 50 volts for 40 min.

2.6. Feasibility assessment of the detection method
Samples for feasibility assessment consisted of SCSMV-infected leaves from the screen house and field samples showing symptoms of SCSMV, i.e. steak mosaic. Leaf samples were subjected for SCSMV detection using 4 methods, i.e. ELISA [9], DIBA [9], conventional PCR [1, 2, 10] and IC-RT-PCR (as described above).

3. Results and discussions

IC-RT-PCR is a method of nucleic acid amplification that combines serological and molecular method. Detection method with a protein approach, such as serological methods, is quite time consuming, relatively low sensitivity and specificity, and particularly insensitive for samples with latent symptoms, very weak infection, and tissue culture plantlets [11]. Whereas the detection method with the nucleic acid approach has a high level of specificity and sensitivity, and also genetic relations between samples can be determined [5]. The very crucial stage in the nucleic acid approach that affects the sensitivity of the test method is RNA isolation, because of the presence of metabolites that can damage RNA [3]. Detection of SCSMV using IC-RT-PCR method was able to amplify specific DNA fragment of 500 bp DNA, although the consistency of the amplification was varied among samples with different dilution rate of $10^0$ to $10^{10}$ (figure 1). Similarly, specific 500 bp DNA fragments were amplified from field samples (figure 2). Further sequencing and sequence analysis of the specific DNA fragments confirmed the SCSMV association. IC-RT-PCR was capable of detecting SCSMV up to $10^4$ dilution [11] whereas in this study, the IC-RT-PCR method can detect SCSMV up to $10^{10}$ dilutions (figure 1).

![Figure 1. Visualization of DNA fragments amplified by IC-RT-PCR using SCSMV AP3/547F primers. DNA templates were diluted serially, $10^0$-$10^{10}$ in columns 1-11, respectively. DNA marker was 1 kb DNA ladder (M). Negative control was a reaction without DNA template control (A).](image1)

![Figure 2. Visualization of DNA fragments amplified by IC-RT-PCR using SCSMV AP3/547F primers. Leaf samples were collected from the field (columns 1 – 6). DNA marker was 1 kb DNA ladder (M). Negative control was a reaction without DNA template control (A).](image2)

To assess the feasibility of IC-RT-PCR method for SCSMV detection, comparison of 4 detection methods was conducted involving conventional RT-PCR, ELISA, and DIBA in addition to IC-RT-PCR. Two out of 5 samples gave a strong reaction to anti-CP SCSMV in ELISA and DIBA (Figure 3C and D). Detection by conventional RT-PCR worked quite well for 2 out of 5 samples, indicated by the amplification of 500 bp DNA fragments. However, unspecific DNA bands of 300 bp were also amplified along with the target 500 bp DNA bands (figure 3A). Detection using IC-RT-PCR gave more specific amplification indicated by the absent of 300 bp unspecific DNA bands. However, the intensity of target 500 bp DNA bands was very weak on samples no. 3, 4, and 5 (figure 3B). This data indicated that the IC-RT-PCR method is more sensitive and specific compared to conventional PCR.

Mosaic disease on sugarcane is caused by 2 major viruses, i.e. SCSMV and Sugarcane mosaic virus (SCMV). The two viruses may induce similar interveinal chlorotic streak mosaic symptoms in infected sugarcane and indistinguishable by foliar symptoms. Both viruses infect sugarcane systemically and once plants are infected the virus can translocate to all plant cells. The risk of widespread incidence of the disease is even very high due to its transmissibility through infected
sugarcane planting materials. The ability of a sensitive, specific and accurate detection method is then very important to prevent disease introduction to previously disease-free regions, especially through infected sugarcane seeds.

![Figure 3](image-url)

**Figure 3.** Comparison of RT PCR (A) IC-RT-PCR (B) ELISA (C) and DIBA (D) for detection of SCSMV from five field samples.

### 4. Conclusion

The IC-RT-PCR method is more sensitive than conventional PCR and can be recommended as a method for indexing sugarcane seeds to produce quality virus-free sugarcane seeds.

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