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Rapid and Sensitive Identification of the SCSMV-infected Sugarcane Based on Immuno-detections

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Abstract: Sugarcane mosaic disease wildly exists in sugarcane-cultivating fields and causes huge impact on sugar industry in China. Sugarcane streak mosaic virus (SCSMV), has been noted to become widely spread in recent years. Although serology-based detection techniques for SCSMV are established, rapid, accurate, and high-throughput detection methods are much less developed. Here, the PAb-^{SCSMV}CP (Polyclonal antibody of ^{SCSMV}CP) was produced and its specificity was tested. There is no serological cross-reaction observed with other viruses that cause sugarcane mosaic disease too. Based on the PAb-^{SCSMV}CP, the immune-detection methods, including double antibody sandwich (DAS) ELISA, dot blotting, and western blotting, were successfully developed. Besides, the colloidal gold immunochromatographic strips were well-prepared and successfully used for rapid and accurate SCSMV detection in the wild field for the first time. Our studies provide serology based high-throughput, rapid, sensitive and quick diagnose methods for SCSMV detection in Chinese sugarcane-cultivating regions at first, which might contribute to the sugarcane mosaic disease control in China.

Key words: sugarcane; mosaic disease; immunological detections; sugarcane streak mosaic virus

快速灵敏的甘蔗线条花叶病毒免疫学检测体系的建立 张 坤[1], 陈春峰[1], 徐红梅[1], 陈佳欢[2], 陈 雯[1], 贺 振[1]* ([1]扬州大学园艺与植物保护学院, 扬州 225009; [2]扬州大学附属医院, 扬州 225009)

摘要:甘蔗花叶病广泛存在于我国甘蔗种植区,严重影响甘蔗产业的高质量发展。近年来甘蔗线条花叶病毒在蔗区肆虐,尽管针对其的血清学检测技术已经建立,但是快速、准确、高通量的检测方法亟待发掘。本研究制备了^{SCSMV}CP 的抗血清,特异性高,与引起甘蔗花叶病的另两种病原(高粱花叶病毒和甘蔗花叶病毒)间没有血清学交叉反应。基于该多克隆抗体,建立了直接抗原包被的 ELISA、斑点杂交、Western blot 和基于多抗的免疫试纸条检测技术。开发的免疫试纸条检测技术能快速、准确、高通量应用于田间病毒鉴定。本文提供了基于血清学的快速、准确、高通量,且便捷的甘蔗线条花叶病毒检测技术,有助于我国蔗区甘蔗花叶病的监测与防控。

关键词:甘蔗; 花叶病; 免疫学检测技术; 甘蔗线条花叶病毒

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Sugarcane (*Saccharum* hybrids spp.), an important economic crop for sugar industry, is widely planted in the most Asian and South America countries, such as China, India, Thailand, Pakistan and Brazil. According to disease surveys conducted in sugarcane-growing regions, an emerging mosaic disease greatly affected the sugar yield in these years [1]. After several years of research, sugarcane mosaic virus (SCMV), sorghum mosaic virus (SrMV), and sugarcane streak mosaic virus (SCSMV) are considered to be the major causal pathogens for mosaic disease [2]. These viruses caused symptoms such as streaks, stripes, or mosaic on leaves and interveinal chlorotic specks. Furthermore, they dramatically reduced the efficiency of photosynthesis and carbon dioxide fixation and eventually influence the carbohydrates accumulation in the cane [2].

SCMV and SrMV belong to the family *Potyvirus*. SCSMV belongs to the family *Poacevirus* [3], and has become widespread in the main Chinese sugarcane-cultivating fields in recent years [4]. SCSMV is a plus single-strand (+ss) RNA virus with approximately 10 kilo-base (kb) genomic RNA and rod-shaped virion [4, 5]. It is transmitted by virus-infected cane during vegetative propagation under natural conditions [1, 6]. Numerous *Poaceae* species could also be infected by artificial sap inoculation, such as maize, millet and sorghum [1, 7]. No vectors have been identified to transmit SCSMV in wild field so far [6, 8]. The genome of SCSMV contains a large open reading frame (ORF) encoding a big polyprotein that could be hydrolyzed to ten mature proteins, which refer to P1, Helper-component protein (HC-Pro), P3, Cytoplasmic inclusions (CI), 6K1, 6K2, Viral protein genome-linked (VPg), Nuclear inclusion a (NIa), Nuclear inclusion b (NIb) and Coat protein (CP) [3].

Sugarcane mosaic disease is mainly caused by SCMV, SrMV and SCSMV. It is hard to distinguish which virus causes the direct induction of final symptoms. For accurate and reliable virus detection, many progresses have been made in these years, such as observation of viral pathogenicity in the sugarcane leaves by microscopy [5], enzyme-linked immunosorbent assay (ELISA) by virus-specific antibodies[9], and reverse transcription polymerase chain reaction (RT-PCR) by virus-specific primers[8, 10]. However, these detection methods still have some limitations in their sensitivity, efficiency, and convenience. It is urgent to develop sensitive, rapid, efficient and high-throughput diagnostic approaches for mosaic disease control in Chinese sugarcane-cultivating fields.

SCSMV encodes a polyprotein and forms mature CP protein after hydrolysis. The mature CP protein contains 281 amino acids with an isoelectric point of 8.7. We detailed analyzed the *cp* gene variability and encoded protein similarities in China[8, 11]. In our study, we cloned the SCSMV *cp* gene and expressed the CP recombinant proteins by *Escherichia coli* Rosetta strain. The prokaryotic expressed CP protein was used as an antigen to immune rabbits for PAb-^{SCSMV} CP preparation. With the PAb-^{SCSMV} CP, we established standard, sensitive and high-throughput detection approaches, such as Western blotting, Dot blotting, and DAS-ELISA. The prepared PAb-^{SCSMV}CP has no serological cross-reaction with other viruses (SrMV and SCMV) that cause sugarcane mosaic disease too. Besides, a colloidal gold immunochromatographic strip was well-prepared and successfully used in the field for the first time. Our study supplied antiserum-based multiple reliable and high-throughput detection approaches for SCSMV in Chinese sugarcane-growing fields.

1 Materials and Methods

1.1 RNA extraction

SCSMV-infected sugarcane leaves were collected from sugarcane-cultivating field in Yunnan province of China. TRIzol Reagent (Invitrogen, USA) was used for the isolation of the total RNA according to the manufacturer's instructions. Genomic DNA contaminations were eliminated by RNase-free

DNase I (TaKaRa, Dalian) before reverse-transcription reaction. The RNA quality was evaluated by 1.2% agarose gel electrophoresis under denaturing conditions (date not shown). The RNA concentration was measured by Nanodrop ND1000 spectrophotometer (Nanodrop technologies, USA). The qualified RNA samples were used as template for cDNA synthesis according to previously described.

1.2 Cloning of the coat protein gene of SCSMV

Total RNA isolated from symptomatic and asymptomatic sugarcane leaves as described above were used as template for reverse transcription reaction. The M-MLV reverse transcriptase (Promege, USA) and primer oligo $(dT)_{18}$ (Invitrogen, USA) were used to synthesize the first strand cDNA according to the instructions. Then the synthetic viral cDNA was treated as PCR template, and pairs of primer ^{SCSMV}CP-F$_{Bam\,H\,I}$/^{SCSMV}CP-R$_{Sac\,I}$ were added to the reaction system to amplify the full-length *cp* gene (Shown in Table 1). The PCR product was purified using the TaKaRa Agarose Gel DNA Purification Kit Version 2.0 (TaKaRa, CA) and ligated to the vector pET28a (Novagen, USA) after double digestion with *Bam* H I/ *Sac* I. The *cp* gene was confirmed by sequencing the constructed vector pET28a-^{SCSMV}CP with T7 sequencing primer.

1.3 Prokaryotic expression and purification of the recombinant CP in *E. coli* Rosetta strain

The correct expression phase of the constructed vector pET28a-^{SCSMV}CP was evidenced and confirmed through sequencing. Then the vector was transformed to the *E. coli* Rosetta strain and colony PCR was performed to screen for positive clones. The positive clone was cultured and used for target recombinant protein expression and purification as previously described with minor modifications [12]. The eluted recombinant ^{SCSMV}CP proteins were concentrated with Ultra-15 filter unit (Millipore, USA) for the rabbit immunization.

1.4 Preparation of the PAb-^{SCSMV}CP

The concentrated ^{SCSMV}CP protein was further dialyzed to remove the salt impurities and quantified by Nanodrop ND1000 spectrophotometer (Nanodrop technologies, USA). To prepare the PAb-^{SCSMV}CP, 2 mg total purified ^{SCSMV}CP recombinant protein emulsified with an equal volume of Freund's complete adjuvant was injected subcutaneously to the New Zealand white rabbits for four times at weekly interval. After 30-days of the first injection, the auricular vein blood was collected for titer determination. About 10 days after the final immunization, total blood was drawn from the heart of the rabbit and centrifuged to obtain the crude serum. Salt fractionation were carried out to precipitate and purify the antibody that existing in crude serum [13]. The obtained precipitate was diluted in normal saline and dialyzed overnight. The dialyzed PAb-^{SCSMV}CP, containing the antibody of ^{SCSMV}CP protein, was mixed with equal volume of 100% glycerol and 0.1% sodium azide, then stored in a -80℃ refrigerator.

1.5 Development of the antibody-based detection methods

The prepared PAb-^{SCSMV}CP was used to perform immunological diagnose of SCSMV in sugarcane-cultivating field of south China. According to our experiments, Western blotting, Dot blotting, ELISA detection, and colloidal gold immunochromatographic strips methods were developed for SCSMV detection.

For Western blot detection, about 0.1 g fresh leaf samples collected from wild field were ground in liquid nitrogen and dissolved in 200 μL 2 × protein extraction buffer (100 mmol · L^{-1} Tris-HCl, pH = 6.8, 20% Glyceral, 4% SDS, 0.2% Bromophenol blue, 5% β-Mercaptoethanol). The homogenates were used for western blot analyses as described previously [12].

For dot blotting detection, 0.1 g fresh leaves collected from sugarcane-cultivating field were ground to powder by liquid nitrogen freezing, and

the powder was dissolved in 2 equal volumes (m/v) of protein extraction buffer (0.01 mol·L^{-1} Citric acid, 0.01 mol·L^{-1} Ammonium citrate, pH=6.5). After 15 min centrifugation at 12 000 *g* under 4℃, the supernatant was transferred to another clear tube. A piece of 20 cm^2 PVDF membrane was prepared and divided to 20 small squares with 1 cm × 1 cm format. The forceps were used to complete all operations instead of touching the membrane with bare hands directly. The membrane was moisturized by 0.02 mol·L^{-1} PBST buffer (0.02 mol·L^{-1} NaCl, 0.02 mol·L^{-1} KH_2PO_4, 0.02 mol·L^{-1} Na_2HPO_4, 0.02 mol·L^{-1} KCl, 0.05% Tween-20) and air dried before loading the samples. Each sample supernatant (5 μL) was spotted on the center of the small squares and air dried. The SCSMV-infected samples and healthy sugarcane samples were treated as positive and negative control respectively. Treatment of the sample-loaded membrane was same with western blotting method as described above. After chromogenic reaction, the membrane was photographed for further quantification analysis.

For ELISA detection, the preparation of the ELISA loading samples was similar to the Dot blotting with minor modifications. Briefly, 0.1 g fresh samples were ground to powder under liquid nitrogen freezing condition and dissolved in 10-times volume of extraction buffer. After 12 000 *g* centrifugation of the sample for 15 min at 4℃, the supernatant was transferred to a clear tube and prepared for next ELISA analyses. The 96-wells polystyrene plates (Costar, USA) were coated by 100 μL/well of extracted supernatant at 37℃ for 3 hours, and each sample was loaded to three wells separately. The next procedures were same with western blotting as above with 2 types antibodies and 3 times washing. Chromogenic reaction was performed by adding 100 μL reaction substrate buffer (1 mg·mL^{-1} pNPP dissolved in 9.7% Diethanolamine at pH for 9.8 or 0.1 mg·mL^{-1} TMB dissolved in solution with 0.1 mol·L^{-1} Citric acid and 0.2 mol·L^{-1} Na_2HPO_4) to each well and incubated for about 30 min at room temperature in the dark. The intensity of the yellow/blue color indicated the reaction activity of each well. Termination reaction buffer (2 mol·L^{-1} NaOH) was added to the well for chromogenic reaction ending. The ELISA reader (Biotek EL X 800, CA) was applied to record the results at OD_{405} or OD_{450}.

For the colloidal gold immunochromatographic strip detection, firstly the gold-PAb conjugated immunochromatographic strip was prepared according to conventional method [14, 15]. For SCSMV detection, the sugarcane extractions were prepared as ELISA. The extractions were added to the sample pad of the strips, and the results were photographed after 30 min. When the test line and control line displayed red color simultaneously, the loaded sample indicated a positive test. When the test line appeared red in color, and the control line without visible band, it indicated that the test was negative. In order to keep the stability of the test, the prepared gold-PAb conjugated immunochromatographic strips were stored at 4℃.

1.6 Dot blot quantification and date analysis

The Image J software (http://imagej.nih.gov/ij/) was used to quantify the intensity of the reaction color in the PVDF membranes according to the instructions. Three Dot blot membranes from independent experiments were measured. Each membrane was measured for three times and the results indicated the average intensity of the appeared dot color.

Readings obtained from the ELISA reader were exported to the Excel software, the average reading of each sample was obtained according to the position on the plates. The criteria for positive ELISA results could be described as follows: the positive control reading minus the blank reading are above two times of the date that negative control reading minus the blank reading.

2 Results

2.1 Cloning and prokaryotic expression of *^{SCSMV}cp* gene

The positive sugarcane samples detected by

Table 1 Primers for SCSMV detection and prokaryotic expression vector construction

Primer name	Primer sequence (5′-3′)[a]	Position[b] /nt	Purpose
^{SCSMV}CP-F$_{BamH\ I}$	GGGCCATGGGAACGCAGCCACCCCAG	8747-8764	Construction of the pET28-CP vector;
^{SCSMV}CP-R$_{Sac\ I}$	GGGGAGCTCTGCTGAGCGCGCCCAAAAT	9588-9570	RT-PCR detection the virus infection;
^{SCSMV}P1-F	CATCGATATGGCTACTATCACTAAGA	218-200	RT-PCR detection of the viral genomic RNA
^{SCSMV}P1-R	CCTCGAGATAAAATACTAAATCTTC	1273-1256	
^{SCSMV}NIb-F	TCCGGATCCATGCACGGAACTGACAAG	7241-7255	RT-PCR detection of the viral genomic RNA
^{SCSMV}NIb-R	TCCGTCGACTTGTCCATCGACCGTTGC	8746-8729	
SCMV-F	GATGCAGGVGCHCAAGGRGG	8412-8431	RT-PCR detection of the SCMV genomic RNA
SCMV-R	GTGCTGCTGCACTCCCAACAG	9335-9315	
^{SrMV}P1-F	CCATCGATATGGCTGGAGCGTGGAAGACT	174-194	RT-PCR detection of theSrMV genomic RNA
^{SrMV}P1-R	GCGTCGACTCAAAAATGATCAATTTCATTAATG	872-851	

[a] Underlined letters represents the restriction enzyme sites.

[b] Numbers shown the corresponding nucleotide sites on genomic RNA of related viruses.

RT-PCR assay (Data not shown) were further used as template, and the pair of specific primer ^{SCSMV}CP-F$_{BamHI}$/^{SCSMV}CP-R$_{Sac\ I}$ (Table 1) was carried out to amplify the *cp* gene product, then the fragment was recycled and cloned to prokaryotic expression vector pET28(a) (Data not shown). The initial vector and the purified *CP* DNA fragments were digested with double restriction-enzyme *Bam*H I and *Sac* I. The positive vector pET28(a)-^{SCSMV}CP was validated by double restriction-enzyme digestion and sequencing. After DNA sequencing, we confirmed that the *cp* gene sequences have already been deposited in NCBI GenBank (JQ954701.1). After double restriction-enzyme digestion for 2 hours, the positive clone released a small fragment similar to the size of the *cp* gene (Data not shown), sequencing result evidenced it is the *cp* gene indeed. Taken together, the construction was correct and was suitable for protein expression.

The constructed pET28(a)-^{SCSMV}CP was transformed to *E. coli* strain Rosetta (DE3)/pLysS and colony PCR was performed to identify the positive clone. The positive clone was cultured in liquid LB media for the protein expression and purification. 1 mmol·L^{-1} IPTG was used for protein expression induction. After stepwise increased imidazole concentration elution assay (Data not shown), we knew that the highest elution efficiency of target recombinant protein was achieved under 200 mmol·L^{-1} imidazole. To avoid protein precipitation, the pH of the protein purification buffer was adjusted to 6.8. Six tubes of elution liquid were obtained and separated by 12.5% SDS-PAGE. According to the coomassie brilliant blue (CBB) staining result of the gel, we chose 200 mmol·L^{-1}, 300 mmol·L^{-1} and 400 mmol·L^{-1} imidazole elution liquid for next operations. Ultra-15 filter unit (Millipore, USA) with molecular weight above 30 kDa was taken to concentrate and further purify the target. The concentrated protein was analyzed by SDS-PAGE and CBB staining (Fig.1-A). The result showed that our purification method is successful and feasible. We took 0.5 mg·mL^{-1} BSA as control to quantify the purified recombinant ^{SCSMV}CP protein. The concentration of our protein is at least two times the amount of the BSA (Fig. 1-B, Compare Lane 2 to 3). These results suggested that our protein purification approach is suitable and the purified ^{SCSMV}CP recombinant protein is relatively pure. The quantity and total amount of the ^{SCSMV}CP protein met the requirement of PAb-^{SCSMV}CP preparation condition and could be taken to immune the New Zealand white rabbits.

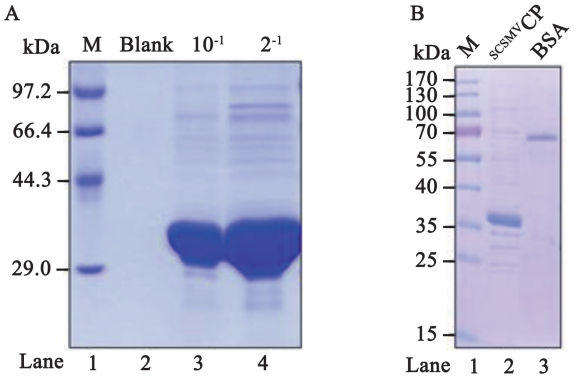

Fig. 1 CBB staining of the purified recombinant ^{SCSMV}CP protein

A: CBB staining of the ten times or 2 times diluted protein. M: Protein marker; Blank: SDS loading buffer; 10^{-1}: 10 times dilution; 2^{-1}: 2 times dilution. B: Quantification of the concentrated ^{SCSMV}CP protein. M: Protein marker; ^{SCSMV}CP: Concentrated recombinant ^{SCSMV}CP protein; BSA: 0.5 mg·mL^{-1} BSA.

2.2 Application and evaluation of the immunological detection approaches for SCSMV detection in Chinese sugarcane-planting regions

The purified recombinant ^{SCSMV}CP protein and the prepared anti-serum was taken as antigen and antibody respectively to perform the ELISA assay. The concentration of ^{SCSMV}CP protein was diluted to 1 μg·mL^{-1}. 50 μL diluted protein was added to coat the 96-well polystyrene plate overnight at 4℃. One percent BSA solution (1% BSA in PBST) was used to block the well, then the diluted protein was captured with stepwise increased diluted PAb-^{SCSMV}CP. The 0.01% horseradish peroxidase conjugated IgG dissolved in 0.02 M PBST buffer was used to capture the first antibody. The readings at OD_{450} were recorded after 10 minutes' chromogenic reaction in 37℃. However, the reading at OD_{450} was still above 0.6 when the dilution ration of the PAb-^{SCSMV}CP reached 1:64 000 (Judgment criterion) (Fig. 2-A). Evaluations of ELISA for the PAb-^{SCSMV}CP suggested that the prepared PAb-^{SCSMV}CP was qualified and could be used for serological based molecular detection method development.

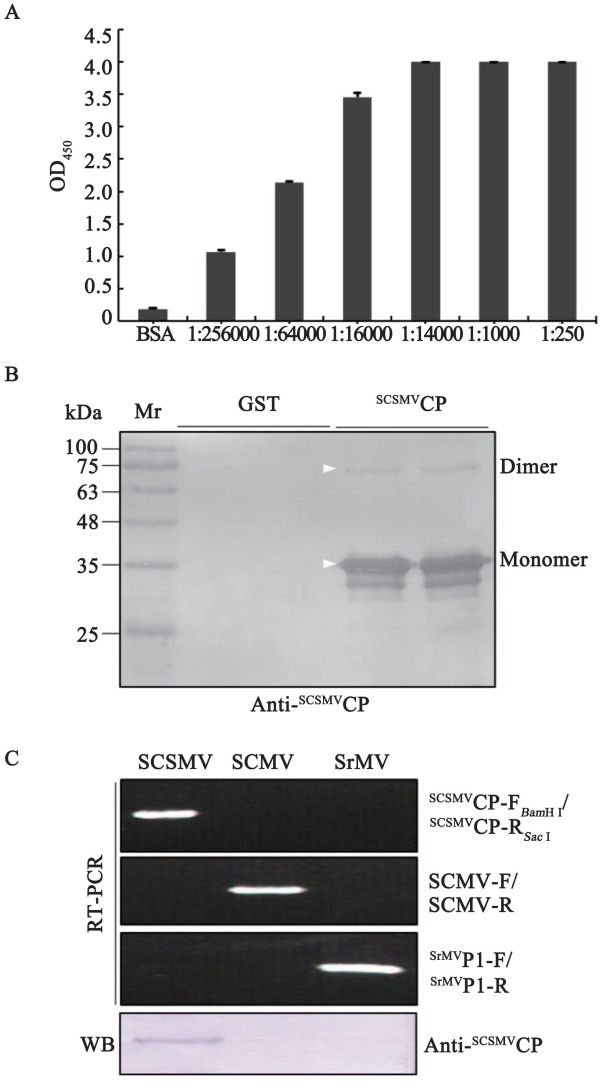

Fig. 2 Evaluation of the titer of prepared PAb-^{SCSMV}CP

A: Titer certification of the PAb-^{SCSMV} CP by ELISA. B: Quality evaluation of the prepared PAb-^{SCSMV} CP by western blotting. GST means the loaded sample was purified GST protein; ^{SCSMV}CP means the purified recombinant protein with N-terminal His-tag. The white triangle shows the target band; C: Specificity validation of the PAb-^{SCSMV}CP for detection of the SCSMV from wild field. The upper three panels show the RT-PCR results with specific pair of primers marked at the right of the gel map. The bottom panel was the western blotting result, and PAb-^{SCSMV} CP was used to detection of the SCSMV.

The purified ^{SCSMV}CP protein was used as antigen for western blot assay to validate the specificity of the prepared polyclonal PAb-^{SCSMV}CP. The purified GST protein and the purified ^{SCSMV}CP-His were taken as the negative and positive samples, respectively. Specific bands (35 kDa) could be easily observed on the membrane in the purified ^{SCSMV}CP-His loaded lane. In addition, another specific band shown 75 kDa was also appeared clearly (Fig. 2-B). We predicted that the upper bands were the dimer form of ^{SCSMV}CP-His, and the bottom bands were the monomer form.

To further determine the specificity of prepared-PAb-^{SCSMV}CP, we collected three samples that were infected by SCSMV, SCMV, and SrMV, respectively. Three pair of specific primers that correlated to SCSMV, SCMV, and SrMV were perform to RT-PCR, and the results identified that the tested samples were single-virus infected (Fig. 2-C, Upper three panels). Then, we extracted the total protein and performed western blotting using the PAb-^{SCSMV}CP. The results showed that the antiserum could only be used for detection of the SCSMV, and not for SCMV and SrMV (Fig. 2-C, Bottom panel). All these results further showed that the prepared PAb-^{SCSMV}CP was qualified and specific for SCSMV detection, and there was no serological cross reaction to SCMV and SrMV.

To further evaluate the specificity of the prepared polyclonalPAb-^{SCSMV}CP, we collected 8 fresh sugarcane samples from the wild field. Each sample represents an independent sugarcane plant, and the samples were ground to powder for further RT-PCR and western blot detection. Sample 1, 3, 4, 5, and 8, exhibited expected specific bands with both RT-PCR and western blot detection (Fig. 3-A, Upper two panel). The results suggested the PAb-^{SCSMV}CP was specific and met the need of the western blotting detection of SCSMV in wild field.

In order toperform high-throughput detection for wild field sugarcane samples with prepared PAb-^{SCSMV}CP, we also developed Dot blot analyses, ELISA, and the gold immunochromatographic strip detection approach. According to the western blotting results in Fig. 3-A, we chose the sample 2 as the negative control, the sample 4 and 5 were selected as the positive control. We ground these samples independently, and each sample represents a sugarcane plant. We named each dot on the membrane. For example, B-1 means the 1st repeat of blank treatment, and repeated for 5 times (the extraction buffer instead of protein extraction), and S4-1 means the 1st repeat of the sample 4, and so on (Fig. 3-B). On the dot blotting membrane, the order of the loaded samples was disorganized, and the intensity of the reddish-brown dots was different (Fig. 3-B). The dots that quantified by ImageJ software were illustrated the by bar graph based on their relative intensity (Fig. 3-C). The relative value of the blank treatment was set for 0, and the negative control was set for 1. On this basis, the relative values of the other samples were calculated. The relative intensity of the dots from the positive samples was several times higher than that of the negative samples (Fig. 3-C). Significant difference was observed between the negative samples and the positive samples through T-test analysis (Fig. 3-C).

The extractions used above were further taken for ELISA analyses. According to the ELISA results, we mapped the bar graph (Fig. 3-D). Equal volume extractions of each sample were loaded to three adjacent wells. At last, the dates of the samples from the same row were averaged and analyzed. Consistent with Dot blot result above, the OD_{405} values of the positive samples (Sample 4 and 5) were much higher than those of the negative samples (Sample 2) (Fig. 3-D).

To develop rapid and convenient SCSMV detection approaches, we prepared the gold-PAb immunochromatographic strips according to the conventional method (Fig. 4-A). The extractions of the 8 samples used in Fig. 3-A were loaded to the sample loading pad on the strip. After 10 minutes, the strips were photographed and stored for further analyses . The

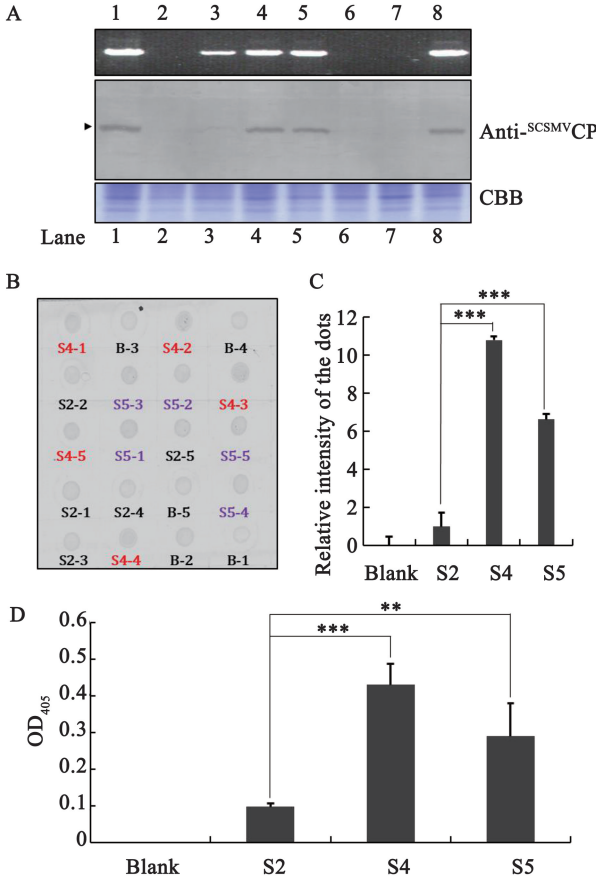

Fig. 3 Application and evaluation of the developed immunological detection methods based on the prepared PAb-^{SCSMV}CP for SCSMV diagnosis in sugarcane-planting regions

A: Detection of the SCSMV-infected samples from Chinese sugarcane-planting regions with RT-PCR and western blot. Total proteins were stained with CBB, which were taken as loading control. B: Evaluation of the developed dot blotting method using known positive and negative samples. The 4th and 5th samples used in Fig. 3-A were taken as positive control, and the 2nd sample for negative control. The name S4-1 means the 1st repeat of the 4th sample that was used in the western blotting detection in Fig. 3-A, and the S4-2 means the 2nd repeat of the 4th sample, The B-1 means the 1st repeat of the blank treatment, each sample was repeated for 5 times, and so on. C: Quantify the intensity of the stained dot in Fig. 3-B by ImageJ software. Error bar SD (Standard Deviation) represent the variation of 5 independent repeats of each sample. D: Evaluation of the developed specific and high-throughput ELISA diagnosis by known positive and negative samples. Similarly, the 4th and 5th samples were taken as positive control, and the 2nd sample for negative control. The sample preparations, date analyses, and statistical methods application were same with dot blotting assay above.

detection results showed the sample 1, 3, 4, 5, and 8, were positive compared to the with the negative control (sample 2) (Fig. 4-B), which were in line with the previously RT-PCR and western blot results. Altogether, these results demonstrated that the high-throughput and rapid approaches based on PAb-^{SCSMV}CP for SCSMV detection in wild field were developed successfully, and it's convenient for SCSMV rapid and accurate detection in wild field.

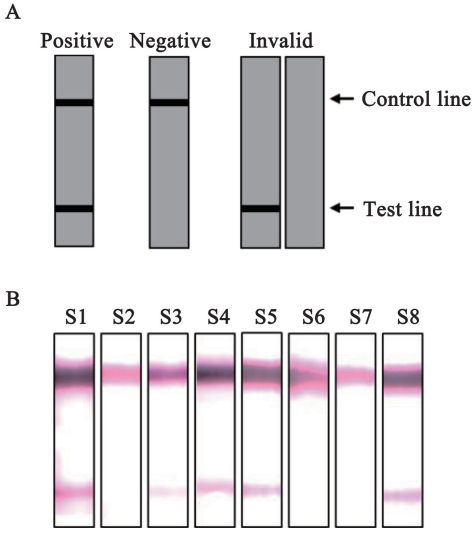

Fig. 4 Application of the developed colloidal gold immunochromatographic strips using known positive and negative samples

A: The judgement criteria of the prepared colloidal gold immunochromatographic strips. B: The 8 fresh samples, which were used in Fig. 3-A, were used for immunochromatographic strips detection again. S1 means the 1st sample collected in Chinese sugarcane-planting areas, and so on.

3 Discussion

The emerging mosaic disease in sugarcane-cultivating field located in south China was caused by the independent or mixed infection of three major viruses (SCMV, SrMV, and SCSMV)[22]. It is hard to identify the real pathogen responsible for disease induction through symptoms observation with our eyes since the mosaic symptom caused by these three

viruses are similar. Hence, molecular detection approaches, such as PCR, RT-PCR, sequencing, RFLP (RT-PCR-based restriction fragment length polymorphism), Western blot, ELISA, DAS-ELISA and antiserum based methods [16~18] were rapidly developed and applied to diagnose these infected plants with mosaic symptom. Meanwhile, the specific detection methods for SCSMV were also developed, such as RT-PCR [8], immune-capture RT-PCR (IC-RT-PCR), duplex-immuno Capture-RT-PCR (D-IC-RT-PCR), one-step real-time RT-PCR[19], direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) [20, 21], double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) [9] reverse transcription loop-mediated isothermal amplification (RT-LAMP) [23], and Dot blotting assays [20].

Among these detection methods, RT-PCR and its derived diagnostic approaches were time consuming and costly. However, the detection results sometimes were unreliable and not suitable for high-throughput detections. The serology-based diagnostic approaches that found to be useful for large scale detection require high-quality and sensitive antibody. Compared with previously described detection methods, we prepared high-quality and specific PAb-^{SCSMV}CP (Fig. 2) that could be used for western blot analysis of sugarcane from wild field (Fig. 3-A). Based on the specific antibody, we developed the high-throughput, sensitive, and rapid detection approaches (DAS-ELISA, Dot blotting, and Gold-PAb immunochromatographic strips). Through experiment tests (Fig. 3-A), the positive samples and the negative samples from sugarcane-cultivating filed could be clearly distinguished by these detection approaches (Fig. 3-B, C, D, and Fig. 4-B). These results once again evidenced these detection approaches to be effective and applicable for SCSMV detection in Chinese sugarcane-cultivating filed.

SCSMV infection of sugarcane could cause mosaic disease. About 10%-15% cane production was reduced in India by the disease[5]. SCSMV widely spread in Chinese sugarcane planting regions recently[11, 19], had posed a big threat to Chinese sugarcane production. Hence, the preparation of high-quality antiserum and the development of high-throughput, sensitive, and rapid detection approaches are urgent for the production of canes in China. SCSMV was transmitted mainly by virus-infected cane cuttings during planting in the sugarcane-cultivating regions. Intensive high-throughput detection of the cane seeds that are used for planting could completely control the viral disease epidemic in sugarcane-cultivating fields. Our research supplied a high-quality antiserum of SCSMV together with three high-throughput, sensitive, and rapid SCSMV detection methods based on PAb-^{SCSMV}CP for Chinese sugarcane-planting fields.

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