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Development of a colloidal gold-based immunochromatographic strip for rapid detection of *Rice stripe virus**

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Abstract: *Rice stripe virus* (RSV) causes dramatic losses in rice production worldwide. In this study, two monoclonal antibodies (MAbs) 16E6 and 11C1 against RSV and a colloidal gold-based immunochromatographic strip were developed for specific, sensitive, and rapid detection of RSV in rice plant and planthopper samples. The MAb 16E6 was conjugated with colloidal gold and the MAb 11C1 was coated on the test line of the nitrocellulose membrane of the test strip. The specificity of the test strip was confirmed by a positive reaction to RSV-infected rice plants and small brown planthopper (SBPH), and negative reactions to five other rice viruses, healthy rice plants, four other vectors of five rice viruses, and non-viruliferous SBPH. Sensitivity analyses showed that the test strip could detect the virus in RSV-infected rice plant tissue crude extracts diluted to 1:20480 (w/v, g/mL), and in individual viruliferous SBPH homogenate diluted to 1:2560 (individual SPBH/μL). The validity of the developed strip was further confirmed by tests using field-collected rice and SBPH samples. This newly developed test strip is a low-cost, fast, and easy-to-use tool for on-site detection of RSV infection during field epidemiological studies and paddy field surveys, and thus can benefit decision-making for RSV management in the field.

Key words: Monoclonal antibody; Colloidal gold-based immunochromatographic strip; *Rice stripe virus*; Rice; Small brown planthopper
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1 Introduction

Rice (*Oryza sativa*) is one of the most important cereal crops worldwide and has an annual global production of 6.50×10^8 t (Wang et al., 2005). Over 90% of rice grain is produced in Asian countries (Wang et al., 2005). Rice viral diseases are common in rice-growing regions and are technically difficult to control, leading to severe losses in global rice production each year (Hibino, 1996). *Rice stripe virus* (RSV) is currently one of the most common rice viruses in China. It is transmitted by the small brown planthopper (SBPH), *Laodelphax striatellus* Fallen, in a persistent circulative-propagative manner (Toriyama, 1986; Falk and Tsai, 1997). It can be transmitted transovarially in SBPH for more than 40 generations (Huo et al., 2014). In recent decades, several outbreaks of RSV were reported in China, Japan, and Korea (Wang et al., 2008; Wu et al., 2009; Otuka

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et al., 2010; Ko et al., 2011). It was reported that the occurrence of RSV in the western region of Japan in 2008 was caused by viruliferous SBPHs migrating from China (Otuka et al., 2010). Although RSV infects mainly rice, it can also infect maize, wheat, oat, foxtail millet, and several grasses in the field (Falk and Tsai, 1997; Lian et al., 2014). The main RSV symptoms on rice plants include leaf chlorosis, necrosis, and plant stunting (Lian et al., 2014).

RSV is a member of the genus *Tenuivirus* and has thin filamentous particles. The genome of RSV consists of four single-stranded RNAs designated RNA1, RNA2, RNA3, and RNA4, in order of decreasing genome size. The genome contains seven open reading frames (ORFs) (Barbier et al., 1992). RNA1 encodes an RNA-dependent RNA polymerase (RdRp) from the viral complementary sense strand. The other three genomic RNAs are all ambisense and each has two non-overlapping ORFs, separated by a non-coding intergenic region (IR), on the opposite strands. The IRs were reported to have transcriptional termination functions (Kakutani et al., 1991; Zhu et al., 1991, 1992; Wu et al., 2013). RNA2 encodes a 22.8-kDa RNA silencing suppressor (P2) from the viral sense RNA2 strand (vRNA2) and a 94.0-kDa polyglycoprotein (PC2) from the viral complementary sense RNA strand (vcRNA2) (Takahashi et al., 1991). RSV vRNA3 encodes a 23.9-kDa major nonstructural protein (NS3), a second viral RNA silencing suppressor (Xiong et al., 2009). The 35.0-kDa coat protein is encoded by vcRNA3 (Hayano et al., 1990; Zhu et al., 1991). RSV vRNA4 encodes a 20.5-kDa disease-specific protein (SP) that interacts with an extrinsic 23.0-kDa protein in the oxygen-evolving complex of photosystem II (PsbP) of rice to enhance viral disease symptoms (Kong et al., 2014). SP also plays a critical role in RSV spread in insect vectors (Wu et al., 2014). RSV vcRNA4 encodes a 32.0-kDa virus movement protein (MP) important for RSV cell-to-cell movement and disease symptom development in rice (Xiong et al., 2008; Xu and Zhou, 2012).

Development of an RSV-specific, sensitive, rapid, low-cost, and high-throughput detection technology is crucial for field management of RSV and for breeding RSV-resistant rice cultivars. Several detection techniques can be used to detect RSV in rice and insect vectors. These methods include enzyme-linked immunosorbent assay (ELISA) (Wang et al., 2004), reverse transcription-polymerase chain reaction (RT-PCR) (Zhang et al., 2008; Li et al., 2015), quantitative RT-PCR (RT-qPCR) (Zhang et al., 2008), and reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Le et al., 2010). However, these methods are laborious and expensive, and require specialized laboratory equipment. For example, RT-PCR is a highly specific and sensitive technique for virus detection. However, it is time-consuming and is not suitable for large-scale field surveys. ELISA is well known as a high-throughput technique for RSV detection. However, it requires highly specific and sensitive antibodies, and is not suitable for on-site field detection (Takahashi et al., 1991; Lian et al., 2014). Several RSV specific antibodies have been used to detect RSV proteins in rice and SBPH tissues (Suzuki et al., 1992; Deng et al., 2012), but no on-site RSV detection technology has yet been documented. The colloidal gold-based immunochromatographic strip assay is currently the quickest technique for plant virus detection. This assay involves antigen-antibody specific binding, colloidal gold labeling, and immunochromatography (Maejima et al., 2014). The method has several advantages over traditional serological assays due mainly to its simplicity, speediness, and limited requirements for working experience and equipment (Frens, 1973). For efficient field studies on RSV epidemiology and detection, in this study, two RSV-specific monoclonal antibodies (MAbs) were produced using purified RSV virion, and a colloidal gold-based immunochromatographic strip was developed using the two newly developed MAbs. Because this strip is RSV-specific, sensitive, cheap, and easy-to-use, we consider that it can be used as an on-site RSV detection tool during field surveys of RSV infection. Results obtained through this on-site detection technique should benefit the prevention and control of RSV infection in paddy fields.

2 Materials and methods

2.1 Viruses, virus purification, and field samples

Rice plants infected with RSV, *Rice black-streaked dwarf virus* (RBSDV), *Southern rice black-streaked dwarf virus* (SRBSDV), *Rice ragged stunt virus* (RRSV), *Rice gall dwarf virus* (RGDV), or *Rice stripe mosaic virus* (RSMV) were collected from rice fields

in China. After identification of the causal virus by RT-PCR and nucleotide sequencing, the different viruses were maintained separately in the laboratory. An RSV-infected and a healthy rice plant were used as the positive and negative controls, respectively. RSV virions were purified from RSV-infected rice plants as described previously (Wang et al., 2004). The quality of purified RSV virion was checked by electron microscopy after negative staining using 0.02 g/mL phosphotungstic acid. The purified RSV virion was then used as the immunogen for anti-RSV MAb production.

A total of 61 rice plant samples and 50 SBPHs were collected from rice fields in the Shandong, Jiangsu, Zhejiang, and Yunnan provinces, China, during the 2017–2018 rice-growing season, and used to test the presence of RSV by the newly developed immunochromatographic strip assay and RT-PCR.

2.2 Preparation and characterization of anti-RSV MAbs

Experiments using animals were performed in accordance with the principles of the Helsinki Accords and approved by the Animal Experimentation Ethics Committee of Zhejiang University, Hangzhou, China. To produce RSV-specific MAbs, five eight-week-old female BALB/c mice were intraperitoneally and subcutaneously immunized with purified RSV virions as described previously (Song et al., 2017). Titers of the serum collected from the immunized mice were determined by an indirect-ELISA. The mouse producing serum with the highest titer was sacrificed and its splenocytes were fused with Sp2/0 murine myeloma cells in polyethylene glycol (1500 Da) as described previously (Zhang et al., 2018). The hybridoma secreting anti-RSV MAb and ascites containing MAb were obtained as described by Chen et al. (2017). Titers of ascites were individually determined by an indirect-ELISA. MAb isotypes were confirmed using an isotyping kit as instructed (Sigma-Aldrich, St. Louis, MO, USA). The specificity and sensitivity of the resulting MAbs were confirmed by western blotting and dot-ELISA as described by Liu et al. (2016) and Chen et al. (2017), respectively. The selected MAbs were purified using the caprylic acid-ammonium sulfate precipitation method (Perosa et al., 1990; Kuang et al., 2013) and stored in small aliquots at −80 °C till used.

2.3 Dot-ELISA

The procedure of dot-ELISA was as described previously (Chen et al., 2017) with some specific modifications. Briefly, a rice plant sample (about 100 mg) was homogenized in about 2 mL 0.01 mol/L phosphate-buffered saline (PBS) at pH 7.4 and an SBPH was crushed in 50 μL PBS with a toothpick. After centrifugation at 3000 r/min for 3 min, supernatant (2.5 μL) from each rice sample or SBPH homogenate was spotted onto a nitrocellulose membrane (GE Healthcare, Bucks, UK). Crude extracts from the healthy or RSV-infected plant tissues, or homogenates from non-viruliferous or viruliferous SBPHs were also spotted onto the membrane and used as the negative and positive controls, respectively. After 10 min air-drying, the membrane was incubated in 0.01 mol/L PBS with 0.05% Tween-20 at pH 7.4 (PBST) containing 0.03 g/mL dried skimmed milk for 30 min followed by 1 h incubation in a diluted MAb solution at room temperature (RT). The membrane was washed three times with PBST and then incubated for 1 h in goat anti-mouse IgG conjugated with alkaline phosphatase (AP) or horseradish peroxidase (HRP) solution (Sigma-Aldrich) at RT. After four washes, the detection signal was visualized by incubating the membrane in a nitro-blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) solution or a 3,3',5,5'-tetramethylbenzidine (TMB) solution (Promega, Madison, WI, USA) for 10–20 min.

2.4 Preparation of colloidal gold-conjugated antibody

Colloidal gold particles (40 nm in diameter) were prepared as described by Wu et al. (2010). After cooling down the colloidal gold solution to RT, 0.2 mol/L potassium carbonate (K_2CO_3) was added to bring the pH to 8.0 and then the solution was stored at 4 °C till used. MAbs 16E6 and 11C1 were purified using the caprylic acid-ammonium sulfate precipitation method (Perosa et al., 1990; Kuang et al., 2013). The optimum MAb concentration for colloidal gold labeling was determined as described by Zhang et al. (2006). For final colloidal gold labeling, 1 mL Milli-Q purified water with 1000 μg purified MAb was added drop by drop into 100 mL of colloidal gold solution and the mixture was stirred gently for 30 min at RT. A total of 10 mL of 10% (0.10 g/mL) bovine serum albumin (BSA) solution containing 0.01 mol/L sodium

borate was added slowly to the colloidal gold/MAb solution until it reached the final concentration of 1% (0.01 g/mL). The mixture was gently stirred for another 30 min at RT, centrifuged at 25000*g* for 30 min at 4 °C , and the colloidal gold-labeled MAb conjugate was washed twice with a 2% (0.02 g/mL) BSA solution as described by Wu et al. (2010). The conjugate was resuspended in 5 mL of 10 mmol/L Tris-HCl buffer (pH 7.4), with 0.01 mol/L sodium borate, 3% (0.03 g/mL) cane sugar, 3% (0.03 g/mL) BSA, and 0.05% (0.5 g/L) sodium azide, before storing at 4 °C till used.

2.5 Preparation of the colloidal gold immunochromatographic strip

Polyester membranes, absorbent papers, sticky bases, and plastic cases were purchased from Shanghai Jieyi Biotechnology, China. Conjugate pads (glass fiber membranes) were purchased from Millipore (Bedford, MA, USA) and soaked in 20 mmol/L phosphate buffer (pH 7.4) containing 2% (0.02 g/mL) BSA, 2% (0.02 g/mL) sucrose, and 0.1% (1.0 g/L) sodium azide, followed by 24 h drying in a 37 °C incubator. Three factors were used to determine the optimal immobilization concentrations of the anti-RSV-coated MAb, the colloidal gold-labeled MAb conjugate, and goat anti-mouse antibodies, as described previously (Wu et al., 2010). Colloidal gold immunochromatographic strips were made using the method described by Wu et al. (2010). Briefly, under optimal conditions, the anti-RSV MAb 11C1 (0.33 mg/mL) and goat anti-mouse antibodies (0.5 mg/mL) were dispensed to the nitrocellulose membrane at the test and control lines, respectively, using the Quanti 3000 BioJets attached to a BioDot XYZ-3000 dispensing platform (Bio-Dot, CA, USA) at a jetting rate of 5.8 μL/cm^2 (the test line and the control line were 5 mm apart) and then dried at 37 °C for 2 h. The colloidal gold-antibody conjugate was dispensed to the treated conjugate pad at a jetting rate of 7.1 μL/cm^2 followed by 2 h incubation at 37 °C to dry. A sample pad, a gold-labeled MAb 16E6 conjugate pad, a nitrocellulose membrane with a test line (T) and a control line (C), and an absorbent pad were glued together tandemly with 2 mm overlap at the conjunction site. These were then placed on a sticky base so that the gold-labeled MAb 16E6 conjugate pad was below the sample pad and overlapped with the nitrocellulose membrane that was positioned at the center of a sticky base. The assembled plate was cut longitudinally with a guillotine cutter to produce strips (60 mm long×3 mm wide). The prepared test strips were packaged inside plastic containers and then stored at RT under desiccated conditions.

2.6 Test procedure of the immunochromatographic strips

For rice samples, about 50 mg of rice leaf tissue was ground in liquid nitrogen and then further homogenized in 0.01 mol/L PBS (pH 7.4) at a ratio of about 1 g tissue to 20–50 mL buffer, or directly ground in a mortar using a pestle and homogenized in 0.01 mol/L PBS without liquid nitrogen. For SBPHs, a single SBPH was placed inside a 0.5-mL Eppendorf centrifuge tube with 150 to 200 μL 0.01 mol/L PBS and then crushed with a toothpick. The strip was laid flat, and then a rice tissue crude extract (no need for centrifugation) or SBPH homogenate (150 to 300 μL) was loaded onto the sample pad. Samples showing two red lines after 5 to 10 min incubation were considered RSV-positive. Samples showing only one red control line were considered RSV-negative. A strip showing the detection line but not the control line was considered an invalid result.

2.7 Detection of RSV infection through RT-PCR

Total RNA was extracted from individual rice samples using TRIzol reagent (Invitrogen). The presence of RSV in rice or insect vector samples was determined using RT-PCR with the primers RSV-CP-F (5'-ATCGGATCCATGGGTACCAACAAGCCAG-3') and RSV-CP-R (5'-TGCTCGAGCTAGTCATCTGCACCTTCTGC-3') specific for the RSV coat protein gene (*CP*; GenBank accession No. JQ927421.1), as previously described (Huang et al., 2013; Xu et al., 2017).

2.8 Detection of RSV in field samples

A total of 61 rice samples and 50 SBPHs were collected from rice fields in the Jiangsu, Shandong, Zhejiang, and Yunnan provinces, China, during the 2017–2018 rice-growing seasons. These samples were tested individually for RSV infection using the above developed dot-ELISA, immunochromatographic strip assay, and RT-PCR methods.

3 Results

3.1 Characterization of RSV-specific MAbs

Thin filamentous particles with the typical morphology of virions in the genus *Tenuivirus* were observed in the purified RSV preparations using a transmission electron microscope (Fig. 1). Two hybridoma lines (16E6 and 11C1) secreting anti-RSV MAbs were prepared using the purified RSV virion as the immunogen. Isotypes of the MAbs were determined to be IgG1, κ light chain. Yields of IgG in the 16E6 and 11C1 ascites were 3.11 and 6.33 mg/mL, respectively, and the titers of the two MAbs were from 10^{-6} to 10^{-7} based on the indirect-ELISA results.

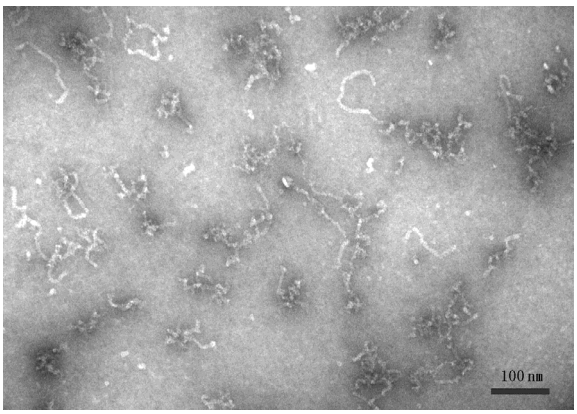

Fig. 1 Electron micrograph of purified *Rice stripe virus* (RSV) virion

3.2 Specificity and sensitivity of MAbs

Western blotting was used to determine the specificities of the two selected MAbs. Results showed that both MAbs reacted specifically with RSV CP (about 35 kDa) from RSV-infected rice leaf tissues or from purified RSV virions (Fig. 2). No positive reaction was observed in the lanes loaded with the rice plant tissue extracts infected with SRBSDV, RBSDV, RRSV, RSMV, or RGDV. Also, no positive reaction was observed in the lanes loaded with healthy rice plant tissue extract (Fig. 2).

Phalanx tests were performed to determine the optimal working concentrations of the MAb and the AP/HRP-conjugated goat anti-mouse IgG for dot-ELISA. Results of three tests showed that the optimal working dilutions for the MAb and the AP/HRP-conjugated goat anti-mouse IgG were 1:5000 and 1:8000 (v/v), respectively. A dot-ELISA was then developed for RSV detection using the optimized antibody dilutions. Using this assay, RSV could be reliably detected in the dots from RSV-infected rice tissues or viruliferous SBPHs. No detection signal was observed in dots from RBSDV-, SRBSDV-, RGDV-, RRSV-, or RSMV-infected or healthy rice plant tissues, or non-viruliferous SBPHs (Figs. 3a and 3b). Further sensitivity analyses indicated that dot-ELISA using MAb 16E6 or MAb 11C1 could detect RSV in 1:20480 (w/v, g/mL) diluted rice plant tissue crude extracts or in 1:5120 (individual SBPH/μL) diluted viruliferous SBPH homogenates (Figs. 3c and 3d).

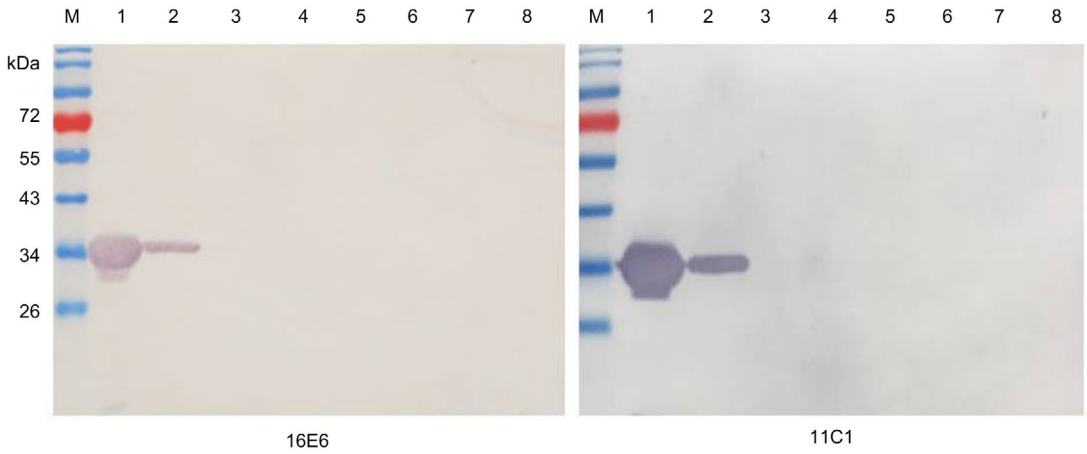

Fig. 2 Specificity analyses of the two MAbs by western blotting

Lane 1 was loaded with purified RSV virion. Lanes 2 to 7 were loaded with rice plant tissue extracts infected with RSV, SRBSDV, RBSDV, RRSV, RSMV, or RGDV. Lane 8 was loaded with an extract from a healthy rice plant and Lane M was loaded with a protein marker

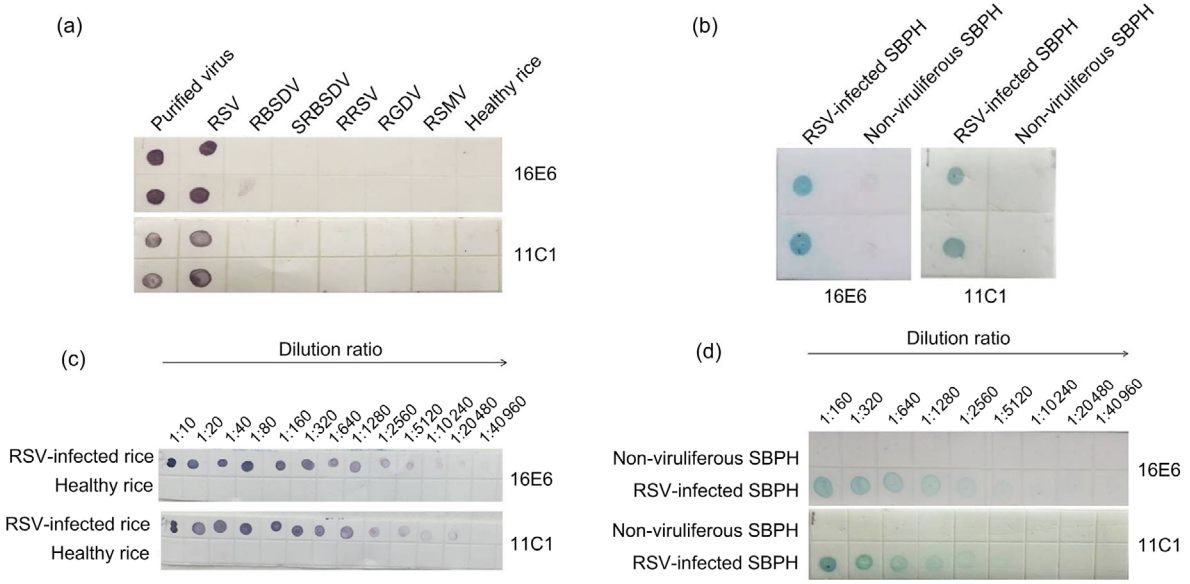

Fig. 3 Specificity and sensitivity analyses of the MAbs by dot-ELISA

(a) Specificity of the two MAbs determined through dot-ELISA using RSV-, RBSDV-, SRBSDV-, RGDV-, RRSV-, or RSMV-infected, or healthy rice plant tissue crude extracts. Two dots in one column were used to represent one sample. Purified RSV virion was used as the positive control. (b) Specificity of the two MAbs determined through dot-ELISA using homogenates from viruliferous or non-viruliferous SBPHs. (c) Sensitivity of the two MAbs determined through dot-ELISA using an RSV-infected rice plant tissue extract diluted from 1:10 to 1:40960 (w/v, g/mL). An extract from a healthy rice plant was used as the negative control. (d) Sensitivity of the two MAbs determined through dot-ELISA using a viruliferous SBPH homogenate diluted from 1:160 to 1:40960 (individual SBPH/μL). A homogenate from a non-viruliferous SBPH was used as the negative control

3.3 Development of the colloidal gold immunochromatographic strip for RSV detection

The prepared colloidal gold solution had a deep red color. Electron microscopy showed that the colloidal gold particles were about 40 nm in diameter, which is suitable for colloidal gold immunochromatographic strip preparation. The optimum MAb IgG concentration for labeling was 1.0 mg/mL, and the colloidal gold-labeled MAb was successfully prepared. Then, an RSV test strip was developed using the method described above.

3.4 Specificity analysis of the colloidal gold immunochromatographic strip for RSV detection

To determine the specificity of the strip, we dropped the strips with rice plant extracts infected with RSV or one of five other tested viruses. Strips dropped with crude extracts from RBSDV-, SRBSDV-, RGDV-, RRSV-, or RSMV-infected or healthy rice plant tissues developed a single red band at the control line, while the strip dropped with an extract from an RSV-infected rice plant tissue developed an additional red band at the test line (Fig. 4a). Specificity of the test strip was then tested using extracts from SBPH, brown planthopper (BPH), white-backed planthopper (WBPH) or leafhoppers *Recilia dorsalis*. Results showed that only the extract from RSV-viruliferous SBPH caused a positive reaction, i.e. two red lines on the strip (Fig. 4b). Extracts from RBSDV-infected or non-infected SBPHs, SRBSDV-infected WBPHs, RRSV-infected BPHs, RGDV- or RSMV-infected leafhopper *R. dorsalis* gave one red line at the control line position, indicating that there were no cross reactions (Fig. 4b).

3.5 Sensitivity of the colloidal gold immunochromatographic strip for RSV detection

Extract from RSV-infected rice tissues was diluted from 1:160 to 1:40960 (w/v, g/mL) with 0.01 mol/L PBS before testing. Homogenate from RSV-infected SBPH was diluted from 1:40 to 1:5120 with the same PBS. The diluted and undiluted extracts were used for testing RSV by strips. Detection results

Fig. 4 Specificity analysis of the developed colloidal gold immunochromatographic strip

(a) Specificity assay of the immunochromatographic strips using crude extracts from RSV-, RBSDV-, SRBSDV-, RGDV-, RRSV-, or RSMV-infected rice plant tissues. The extract from a healthy rice plant tissue was used as a negative control. (b) Specificity assay of the immunochromatographic strips using homogenates from RSV- or RBSDV-infected SBPHs, SRBSDV-infected WBPHs, RRSV-infected BPHs, RGDV- or RSMV-infected leafhopper *Recilia dorsalis*. Extract from non-viruliferous SBPH was used as the negative control

indicated that RSV could be detected in RSV-infected rice crude extracts diluted up to 1:20480 (w/v, g/mL) or in RSV-viruliferous SBPH homogenate diluted up to 1:2560 (individual SBPH/μL), as shown in Fig. 5.

3.6 Detection of RSV in field-collected rice plants or SBPHs

A total of 61 rice plant samples and 50 SBPHs collected from paddy fields in the Jiangsu, Zhejiang, Shandong, and Yunnan provinces of China were screened for the presence of RSV by the developed dot-ELISA, colloidal gold immunochromatographic strip, and RT-PCR methods. Results of the dot-ELISA assay showed that 50 tested rice samples and 11 tested SBPHs were infected with RSV (Figs. 6a and 6d, Table 1). These findings agreed with the results obtained through the strip detection method (Figs. 6b and 6e, Table 1). In addition, these serological assay results were in agreement with the results obtained through RT-PCR (Figs. 6c and 6f, Table 1).

4 Discussion

Rice virus diseases are technically difficult to control and often cause serious reductions in rice yields in many countries. Among the reported rice viruses, RSV often causes serious damage to rice production in Asia (Huang et al., 2013). Thus, development of an on-site, sensitive, and high-throughput detection technology is a key objective for the design of strategies for managing this viral disease.

Serological detection techniques are often used to detect viruses in plant tissues due mainly to their low cost, high specificity, sensitivity, ease of use, and fast and high-throughput capability (Liu et al., 2016). Earlier, our laboratory reported RSV-specific MAbs and a dot-ELISA assay using a MAb developed for RSV detection (Wang et al., 2004). That assay has now been widely applied in rice fields in China. However, because the dot-ELISA assay is relatively complicated to use and takes about 2 h to complete,

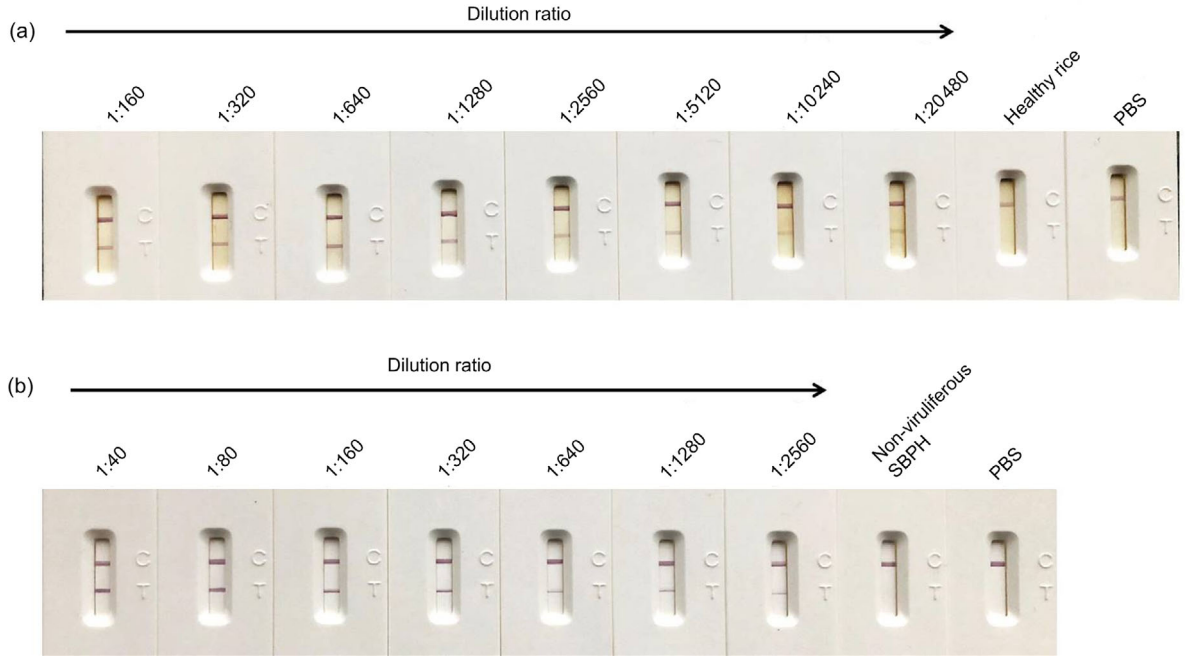

Fig. 5 Sensitivity analysis of the developed colloidal gold immunochromatographic strip

(a) Sensitivity assay using an RSV-infected rice leaf extract. The number above each strip indicates the dilution of the sample. An extract from healthy rice leaf tissues and PBS only were used as negative controls. (b) Sensitivity assay using an RSV-infected SBPH extract. The number above each strip indicates the dilution of the sample. An extract from non-viruliferous SBPH and PBS only were used as negative controls

a quicker and simpler assay using a colloidal gold immunochromatographic strip is required to be developed. Our goal was to enable researchers and growers to detect RSV infection in rice samples or SBPHs in an accurate, rapid, and low-cost manner.

The usefulness of a serological detection method depends largely on the quality of the antibody used in the assay. Therefore, preparations of highly sensitive and specific antibodies are critical for establishing reliable serological methods. In this study, two highly sensitive and specific anti-RSV MAbs (16E6 and 11C1) were successfully generated using purified RSV virion as the immunogen. Both MAbs could react strongly with RSV in rice plant tissues or viruliferous SBPH homogenates, but not with rice plant tissues infected with RBSDV, SRBSDV, RGDV, RRSV, or RSMV. No positive reactions were observed when healthy rice extracts or non-viruliferous SBPH homogenates were used. Using a dot-ELISA and serially diluted RSV-infected rice plant tissue crude extracts or SBPH homogenates, it was determined that RSV could be reliably detected in rice plant tissue extracts diluted at 1:20480 (w/v, g/mL) or in individual viruliferous SBPH homogenates diluted at 1:5120 (individual SBPH/μL). To our knowledge, these two MAbs are the most sensitive and specific anti-RSV MAbs reported.

Using these two MAbs, we have now developed a colloidal gold immunochromatographic strip. The principle of the strip test is as follows: crude extracts of rice plants or homogenates of SBPHs are loaded onto the sample pad of the strip and diffuse throughout the strip through capillary action. If the sample is infected with RSV, virion will bind with anti-RSV MAb 16E6 conjugated with colloidal gold (gold/RSV MAb 16E6) to form colloidal gold/RSV MAb 16E6:RSV virion complex in the conjugated pad. This complex will then migrate into the nitrocellulose membrane. When this colloidal gold/RSV MAb 16E6:RSV virion complex is captured by another anti-RSV MAb 11C1 at the test line position, colloidal gold/RSV MAb 16E6:RSV virion:RSV MAb 11C1 complex will form and produce a visible red band at the test line position. The excess gold/RSV MAb

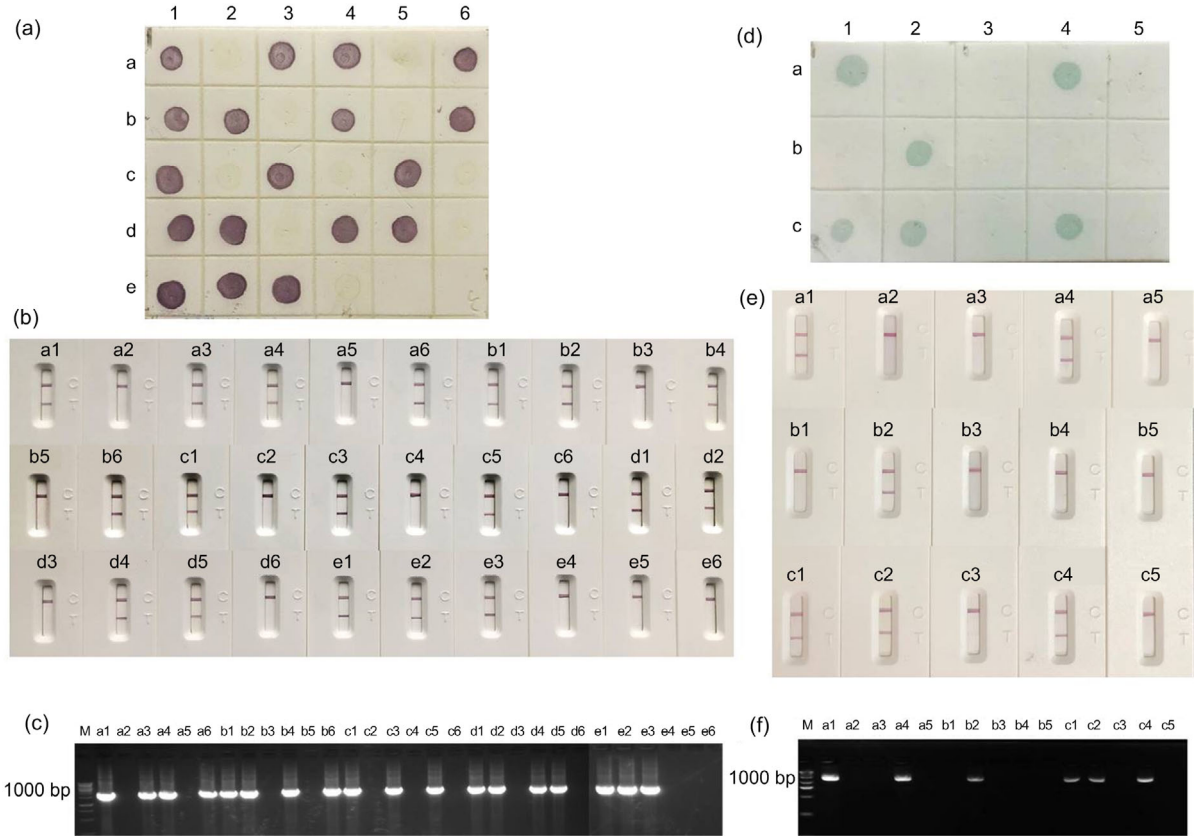

Fig. 6 Detection of RSV in field-collected samples by dot-ELISA, the strip test, and RT-PCR

(a–c) Detection of RSV in field-collected rice samples by dot-ELISA, the strip test, and RT-PCR, respectively. e3 and e4 refer to samples from an RSV-infected and a healthy rice plant, used as a positive and a negative control, respectively. (d–e) Detection of RSV in field-collected SBPH samples by dot-ELISA, the strip test, and RT-PCR, respectively. c4 and c5 refer to samples from an RSV-infected and a non-viruliferous SBHP, used as a positive and a negative control, respectively

Table 1 Detection of RSV infection in field-collected rice and SBPH samples through dot-ELISA, strip test, and RT-PCR methods

Sample	Positive number/detected number		
	Dot-ELISA	Strip test	RT-PCR
Rice sample	50/61	50/61	50/61
SBPH	11/50	11/50	11/50

16E6 without RSV virion will continue to migrate and finally be captured by goat anti-mouse IgG at the control line position to form a colloidal gold/RSV MAb 16E6:anti-mouse IgG complex and produce a second red color band at the control line position. The remaining solution will continue to move along the strip and accumulate in the absorption pad. If the sample has no RSV virion, a single red color band will be shown at the control line position.

The developed strip did not cross react with five other rice viruses tested, or with healthy rice plant or non-viruliferous SBPH homogenates. The sensitivity results showed that the test strip was still effective even when diluting the RSV-infected rice plant tissue samples to 1:20480 (w/v, g/mL), and diluting the RSV-infected individual vector homogenate to 1:2560 (individual SBPH/μL). The specificity and sensitivity of this strip were also validated through RT-PCR. Therefore, the test strip developed in this study is highly specific and sensitive for RSV detection in field-collected rice plant samples or in SBPHs. A test strip kit will contain a strip, a plastic dropper, extract buffer, an Eppendorf tube, a toothpick, and a mortar and pestle. Thus, this strip test does not need any complex equipment or additional reagents. Furthermore, it is well known that the colloidal gold immunochromatographic strip test is the simplest and

fastest detection technique. So, we highly recommend this strip for RSV epidemiological studies and RSV detection.

In conclusion, we have successfully generated two RSV-specific MAbs and used them to establish a colloidal gold immunochromatographic strip for RSV on-site detection. Because this strip test can be done in 5–10 min without need of special instruments or skilled personnel, we consider that it will benefit future investigations of RSV occurrence in rice fields and the design of proper prevention and control strategies for this devastating disease in China and other countries.

Contributors

De-qing HUANG and Rui CHEN prepared the MAbs and developed the colloidal gold immunochromatographic strip. Ya-qin WANG performed the RT-PCR detection. Jian HONG, Xue-ping ZHOU, and Jian-xiang WU conceived the study, participated in its design, and helped draft the manuscript. All authors read and approved the final manuscript. Therefore, all authors have full access to all the data in the study and take responsibility for the integrity and security of the data.

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Compliance with ethics guidelines

De-qing HUANG, Rui CHEN, Ya-qin WANG, Jian HONG, Xue-ping ZHOU, and Jian-xiang WU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：一种快速检测水稻条纹病毒的胶体金免疫层析试纸条的研制

目　的：制备一种检测水稻植物和传毒介体灰飞虱中水稻条纹病毒（RSV）的胶体金免疫层析试纸条，为水稻条纹叶枯病的田间调查和检测以及预测预警提供快速、实用的检测试剂。

创新点：利用制备的 RSV 单克隆抗体，首次制备了能在 5~10 min 内快速、准确、灵敏、特异地检测水稻植物和灰飞虱体内 RSV 的胶体金免疫层析试纸条。

方　法：以差速离心方法提纯的 RSV 病毒粒子作为免疫原免疫 BALB/c 小鼠，通过杂交瘤技术获得了高度特异和灵敏的 RSV 单克隆抗体。采用柠檬酸钠还原氯金酸的方法制备胶体金并标记一个 RSV 单克隆抗体，另一个 RSV 单克隆抗体和羊抗鼠抗体分别包被到硝酸纤维素膜的检测线和质控线，将吸水滤纸制成的样品垫、RSV 免疫胶体金垫、结合有 RSV 单抗和羊抗鼠抗体的硝酸纤维素膜和吸水纸依次粘贴到聚氯乙烯（PVC）胶板上研制检测水稻植物和传毒介体灰飞虱中 RSV 的胶体金免疫层析试纸条。对田间样品进行 RSV 检测，分析试纸条检测 RSV 的有效性。

结　论：利用杂交瘤技术获得了 2 株 RSV 单克隆抗体（16E6 和 11C1），胶体金标记 16E6 单克隆抗体包被在聚酯膜制成的结合垫上，以 11C1 单克隆抗体和羊抗鼠抗体分别包被到硝酸纤维素膜的检测线和质控线，制成能在 5~10 min 内快速、特异、灵敏、准确地检测田间水稻及单头灰飞虱样品中 RSV 的胶体金免疫层析试纸条。试纸条检测感染 RSV 的水稻植株和灰飞虱呈阳性反应，而检测健康水稻和感染其它 5 种常见水稻病毒的植株及其传毒介体呈阴性反应，且试纸条检测 RSV 感染水稻植物组织的灵敏度达到 1:20480 倍稀释（w/v, g/mL），检测携带 RSV 单头灰飞虱的灵敏度达到 1:2560 倍稀释（单头灰飞虱/μL）。田间样品检测结果发现，试纸条检测结果与反转录聚合酶链反应（RT-PCR）的检测结果一致，表明制备的试纸条可有效地用于田间 RSV 的检测，从而为水稻条纹叶枯病的诊断、监测预警、抗病育种、流行病学研究及科学防治提供技术和物质支撑。

关键词：单克隆抗体；胶体金免疫层析试纸条；水稻条纹病毒；水稻；灰飞虱