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Three highly sensitive monoclonal antibody-based serological assays for the detection of tomato mottle mosaic virus

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Abstract

In recent years, tomato mottle mosaic virus (ToMMV) has become one of the most important viral pathogens affecting solanaceous crop production in Yunnan, Hainan, and Shandong provinces of China, often causing huge yield reductions. To provide farmers and vegetable industry with reliable and easy-to-use ToMMV detection methods, we immunized BALB/c mice with purified ToMMV and obtained six hybridoma cell lines (i.e., 2D6, 9C12, 26A10, 3A4, 23A4 and 17B11) that secrete anti-ToMMV monoclonal antibodies (MAbs) through the hybridoma technology. Using these MAbs as the detection antibody, we developed three serological assays: antigen-coated-plate enzyme-linked immunosorbent assay (ACP-ELISA), dot enzyme-linked immunosorbent assay (dot-ELISA) and tissue print enzyme-linked immunosorbent assay (tissue print-ELISA) for ToMMV detection. Our test results showed that these three newly developed serological methods can be used to specifically detect ToMMV infection in plant samples, but not tobacco mosaic virus, tomato mosaic virus, cucumber green mottle mosaic virus and cucumber mosaic virus. Sensitivity analyses further showed that ACP-ELISA and dot-ELISA can be used to detect ToMMV infection in plant crude extracts diluted at 1:81,920 and 1:40,960 (weight/volume, g/mL), respectively. Surprisingly, the detection limit of the developed dot-ELISA was 26 times higher than that of traditional RT-PCR. Using field-collected plant samples, we have demonstrated that these three new serological methods are accurate and easy-to-use for large-scale detection of ToMMV in fields.

Keywords: Tomato mottle mosaic virus, Monoclonal antibody, Serological assay, Virus detection

Background

Tomato (*Solanum lycopersicum*) is an important vegetable crop with high economic values. However, tomato plants are often infected by a variety of viruses, including plant RNA and DNA viruses. For example, tomato mottle mosaic virus (ToMMV) has been reported to infect tomato and other solanaceous crops in recent years, causing serious damages to their production (Padmanabhan et al. 2015). ToMMV was first reported in Mexico in 2013 and is now prevalent in tomato, eggplant, pepper and tobacco fields worldwide (Li et al. 2013). In China, ToMMV has been found in tomato fields in Yunnan, Hainan, Hebei, Henan and Liaoning provinces, and also in Tibet Autonomous Region, resulting in serious yield losses (Li et al. 2017; Zhan et al. 2018).

ToMMV is a new species of the genus *Tobamovirus* in the family *Virgaviridae*. Like tobacco mosaic virus (TMV), the ToMMV genome consists of a single-stranded positive-sense RNA, with a methylguanosine [m7G(5')pppG] cap and a tRNA-like structure at

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its 5' and 3' ends, respectively (Li et al. 2013). ToMMV genomic RNA contains four open reading frames (ORFs), encoding two replication-related proteins of 126 and 183 kDa, a move protein (MP) of 29.8 kDa and a coat protein (CP) of 17.5 kDa (Li et al. 2013; Sui et al. 2017). ToMMV shares the highest nucleotide sequence similarity (84.3%) with tomato mosaic virus (ToMV) (Li et al. 2013; Li et al. 2017). The deduced amino acid sequences of the 126 and183 kDa replication-related proteins, MP and CP of ToMMV share 94.3%, 94.7%, 81.4% and 91.4% identities with the corresponding proteins of ToMV, respectively (Nagai et al. 2019; Rodriguez-Mendoza et al. 2019).

ToMMV particle is about 300–310 nm long and 18 nm wide, and contains a single 6.3–6.5 kilobase (kb) RNA molecule (Li et al. 2018). ToMMV is highly stable and can survive for many years in infected plant debris or on seed surface. This virus can infect many other solanaceous crops, including eggplant and pepper. Common symptoms of ToMMV-infected plants are leaf chlorosis and mottling, and plant stunting (Padmanabhan et al. 2015). ToMMV can be transmitted over a long distance through contaminated seeds. Once infected, ToMMV can be readily transmitted to the surrounding plants in the same field through culture practices (Chen et al. 2018; Zhan et al. 2018).

Currently, three diagnostic methods (i.e., electron microscopic observation, reverse transcription polymerase chain reaction (RT-PCR) and serological assays) can be used to detect ToMMV infection in plants. Among them, RT-PCR is the most reliable method for the detection of ToMMV (Li et al. 2013). In a previous report, RT-PCR was used to detect tobacco mosaic virus (TMV), tomato mosaic virus (ToMV) and ToMMV simultaneously in plants (Sui et al. 2017). In a separate report, Northern blot with digaoxin-labeled ToMMV-specific cDNA probes was shown to be effective for ToMMV detection (Ambrós et al. 2017).

Serological methods are currently widely used to detect virus infection in plants, due mainly to their high sensitivity and specificity, fast detection speed and simple operation (Chen et al. 2018; Guo et al. 2020; Zhang et al. 2020). For example, a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) kit manufactured by Agdia Inc. (Elkhart, USA) has been widely used to detect TMV infection in plants. However, this detection kit is unable to distinguish between TMV and ToMMV infection in infected plants. Zhan et al. (2018) used anti-TMV monoclonal antibodies (MAbs) to detect TMV and ToMMV, and then used RT-PCR to further determine the infection by ToMMV. The genomes of ToMMV and TMV share a high nucleotide identity (over 80%) (Li et al. 2013), which results in a powerful serological cross-reactivity between these two viruses. Thus far, there have been no reported serological methods that can specifically detect ToMMV. Since the MAbs produced in this study are highly specific to ToMMV, they can be used to distinguish ToMMV infection from TMV or other tobamovirus infections in plants. Thus, the serological methods described here can ensure reliable detection of ToMMV in plant samples during large-scale field surveys to provide crucial information on disease occurrence, which is critical for the prevention and management of ToMMV disease.

Results

ToMMV virion purification

ToMMV infection in tomato plants used for virion purification was confirmed by RT-PCR (Fig. 1a). ToMMV virions were then purified from ToMMV-infected tomato leaf tissues through differential centrifugations. Numerous baculovirus-like particles of 300–310 nm long and 17–19 nm wide were observed in the purified virus preparation under an electronic microscope. The morphology of ToMMV virions was similar to that of other tobamoviruses (Fig. 1b).

Production and characterization of ToMMV-specific MAbs

On the third day after the fourth immunization, splenocytes were isolated from the immunized BALB/c mice and used for hybridoma preparation. Through cell fusion and selection, antibody detection and cell cloning, six hybridoma cell lines (2D6, 9C12, 26A10, 3A4, 23A4 and17B11) were found to secret ToMMV-specific MAbs. These hybridomas were then injected individually and intraperitoneally into the original primed BALB/c mice to produce ascites containing MAbs. Isoforms and subclasses of these six MAbs were identified as IgG2a, kappa light chain (Table 1). Titers of the six ascites were determined to be up to 10^{-6} through an indirect ELISA (Table 1). Yields of IgG in the six ascites ranged from 4.36 to 6.38 mg/mL (Table 1). Western blot analysis revealed that the six MAbs reacted strongly and specifically with a ~ 18 kDa and a ~ 36 kDa protein from ToMMV-infected tomato plant tissues (Fig. 2). No such signal was detected in samples prepared from ToMV-, TMV- or CMV-infected plants, or from uninfected tomato plants (Fig. 2). According to the molecular weight of the detected protein bands, we consider that the MAbs prepared in this study are specific for ToMMV CP (~ 18 kDa), and the ~ 36 kDa protein band is most likely to represent the dimers of ToMMV CP (Fig. 2). Another smaller protein band produced by ToMMV-infected sample is likely to be the degraded products of ToMMV CP.

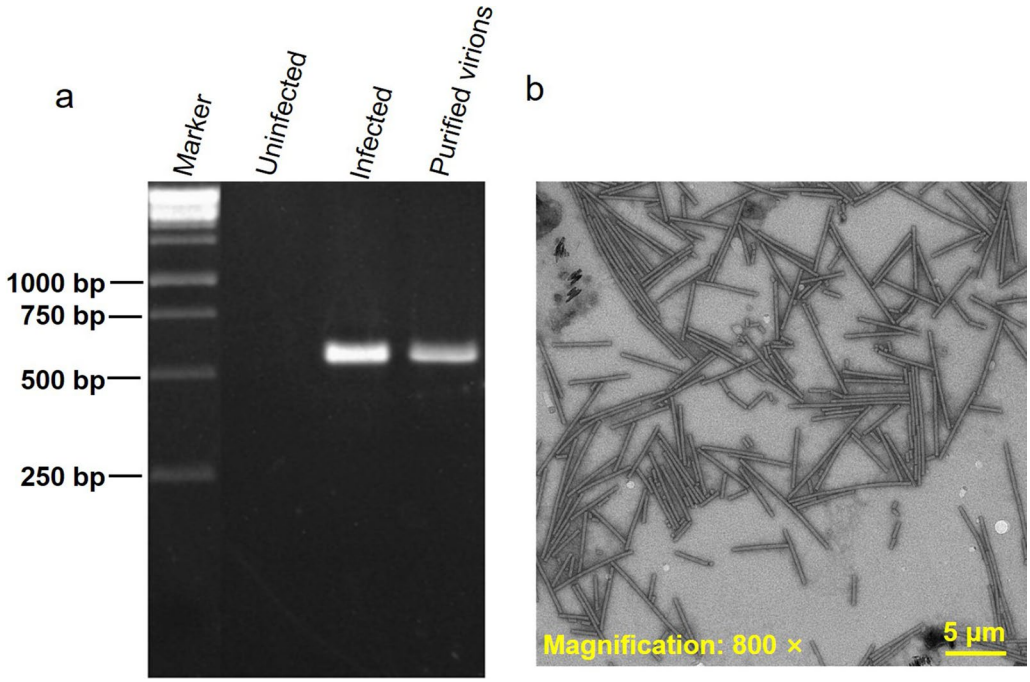

Fig. 1 RT-PCR identification and electron microscopic observation of purified ToMMV virions. **a** The ToMMV infection in tomato plants used for virion purification was confirmed by RT-PCR. **b** Electron micrograph of purified ToMMV virions. ToMMV virions were purified from ToMMV-infected tomato leaf tissues. The negative staining of virus particles was performed using 1% phosphotungstic acid solution, the samples were subsequently examined and imaged under an electron microscope. Bar = 5 μm

Table 1 Characteristics of the ToMMV-specific MAbs produced in this study

MAbs	Isotype	Titre	IgG yield (mg/mL)
2D6	IgG_{2a}, κ chain	10^{-8}	6.38
9C12	IgG_{2a}, κ chain	10^{-8}	5.92
26A10	IgG_{2a}, κ chain	10^{-6}	4.36
3A4	IgG_{2a}, κ chain	10^{-7}	5.28
23A4	IgG_{2a}, κ chain	10^{-7}	4.44
17B11	IgG_{2a}, κ chain	10^{-7}	5.54

Detection of ToMMV infection using ACP-ELISA

The results of three independent tests with serially diluted antibodies revealed that ToMMV could be reliably detected in the infected tomato extracts through ACP-ELISA using anti-ToMMV MAb at a dilution of 1:7000 (v/v) and AP-conjugated goat anti-mouse IgG second antibody at a dilution of 1:8000 (v/v). Cross reaction assay results showed that the six anti-ToMMV MAbs reacted strongly and specifically with ToMMV, but not with TMV, ToMV, cucumber green mottle mosaic virus (CGMMV) and cucumber mosaic virus (CMV). Again, no positive reaction was observed in extracts from uninfected tomato tissues (Fig. 3a). Using a series of two-fold diluted ToMMV-infected or uninfected tomato plant extracts, we determined that ACP-ELISA based on MAb 2D6, 9C12, 3A4, 23A4 or 17B11 can detect ToMMV infection in plant extracts diluted up to 1:81,920 (w/v), while ACP-ELISA based on MAb 26A10 can detect ToMMV infection in plant extracts diluted up to 1:40,960 (w/v) (Fig. 3b).

Detection of ToMMV infection through dot-ELISA and tissue print-ELISA

To develop dot-ELISA and tissue print-ELISA for ToMMV detection, three independent tests using serial dilutions of the six MAbs and AP-conjugated goat anti-mouse IgG second antibody were performed. The results showed that the optimal working dilutions of all six MAbs and AP-conjugated goat anti-mouse IgG second antibody for dot-ELISA and tissue print-ELISA were 1:6000 and 1:8000 (v/v), respectively. Using these antibodies at the optimized working dilutions, dot-ELISAs and tissue print-ELISAs for ToMMV detection were established. To test the specificities of dot-ELISAs, plant extracts were prepared respectively from ToMMV-, TMV-, ToMV-, CMV- and CGMMVV-infected plant samples. The extract from uninfected tomato samples was used as a

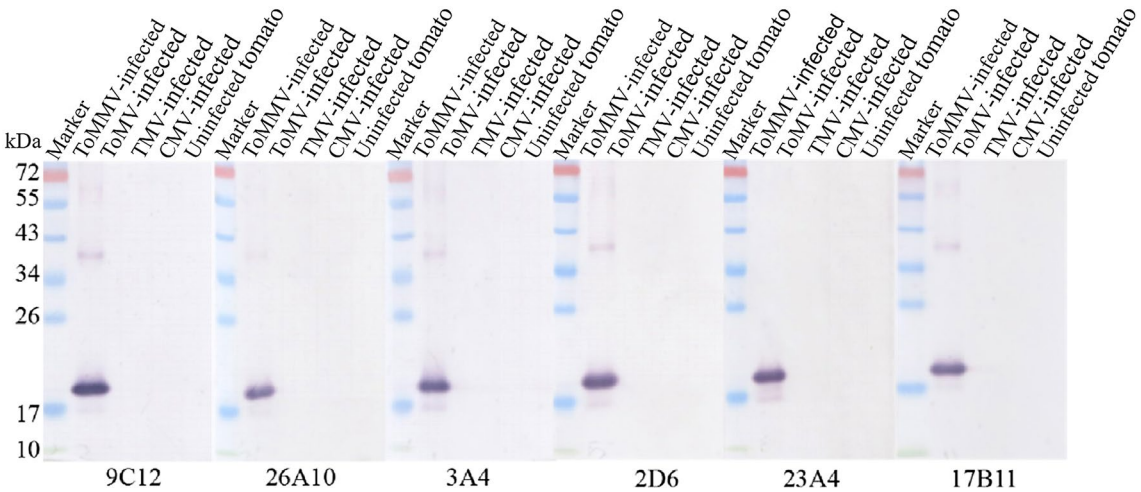

Fig. 2 Western blot analyses of anti-ToMMV MAbs. Extracts were prepared from ToMMV-, ToMV-, TMV- or CMV-infected plant tissues and then loaded individually into SDS-PAGE gel wells followed by electrophoresis. The sample from healthy (uninfected) tomato leaves was used as a negative control. The MAbs were diluted 1:5000 (v/v) in PBS

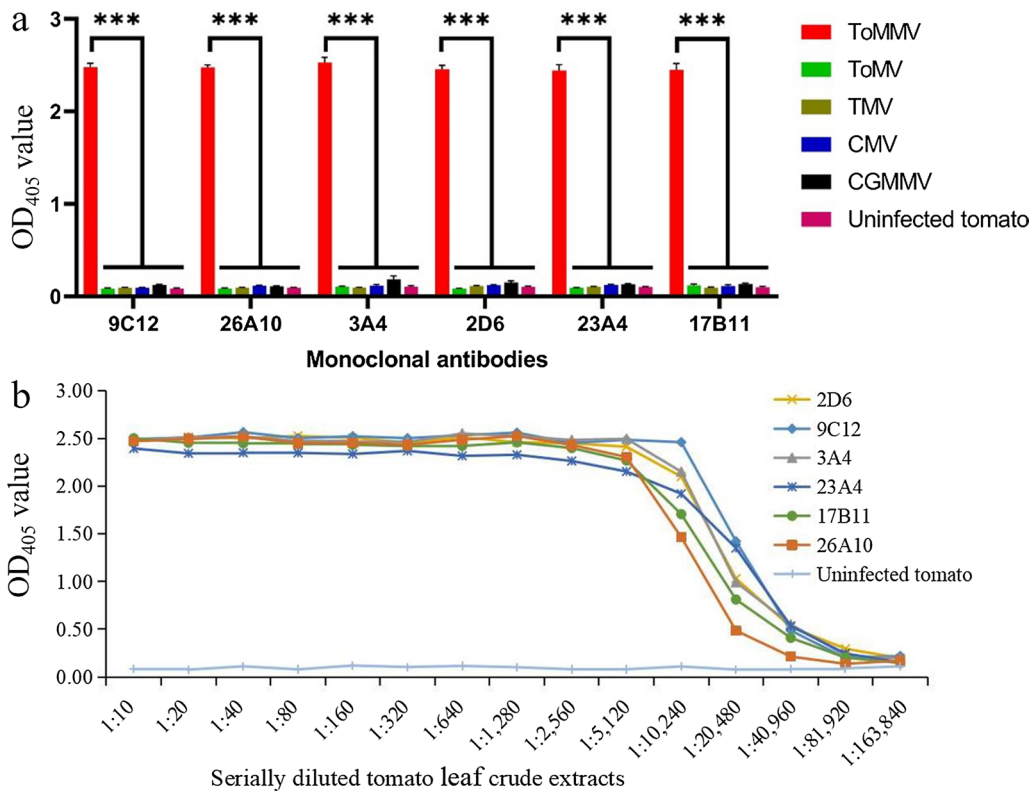

Fig. 3 Specificity and sensitivity analyses of the six MAbs using ACP-ELISA. Extracts were prepared from ToMMV-, ToMV-, TMV-, CMV- or CGMMVV-infected plant tissues, with the extract from uninfected tomato samples as a negative control. The extracts were diluted 1:30 (w/v) prior to ACP-ELISA (**a**) or serially diluted in the coating buffer (1:10 to 1:163,840 w/v) prior to ACP-ELISA (**b**). The optical density (OD) at 405 nm was read at 30 min post adding the substrate and expressed as mean ± standard deviation (SD) of three biological replications. ***$P \leq 0.01$

negative control. The results of three independent tests showed that all six dot-ELISAs based on the prepared MAbs could specifically detect ToMMV (Fig. 4a). To analyze the sensitivities of dot-ELISAs, we prepared extracts from ToMMV-infected and uninfected tomato samples, and serially diluted them from 1:20 to 1:81,920 (w/v) in 0.01 mol/L PBS (pH 7.4). The diluted extracts were then used as the detection samples to determine the sensitivities of dot-ELISAs. Our analysis results showed that ToMMV could be detected in ToMMV-infected extracts diluted at 1:20,480 (w/v) or in 0.12 μg of infected plant tissues by dot-ELISA based on MAb 2D6, 9C12 or 3A4, and in ToMMV-infected extract diluted at 1:10,240 or in 0.24 μg of infected plant tissues by dot-ELISA based on MAb 23A4, 17B11 or 26A10 (Fig. 4b). To compare the detection sensitivity between dot-ELISAs developed in this study and conventional RT-PCR, total RNA (30 μL) extracted from 100 mg of ToMMV-infected tomato plants was serially two-fold diluted from 1:4 to 1:2048 (v/v) in DNase/RNase free ddH_2O, and 1μL of each RNA dilution was subsequently used to determine the sensitivity of RT-PCR. The results showed that ToMMV could be detected in the 1:1024 (v/v) diluted total RNA isolated from infected tomato plants or in 3.26 μg of infected plant tissues (Fig. 4c). Surprisingly, the detection limit of the developed dot-ELISA was 26 times higher than that of RT-PCR.

To determine whether tissue print-ELISAs can also be used to detect ToMMV infection in tomato plants, stems were harvested from ToMMV-, ToMV-, TMV-, CMV-,

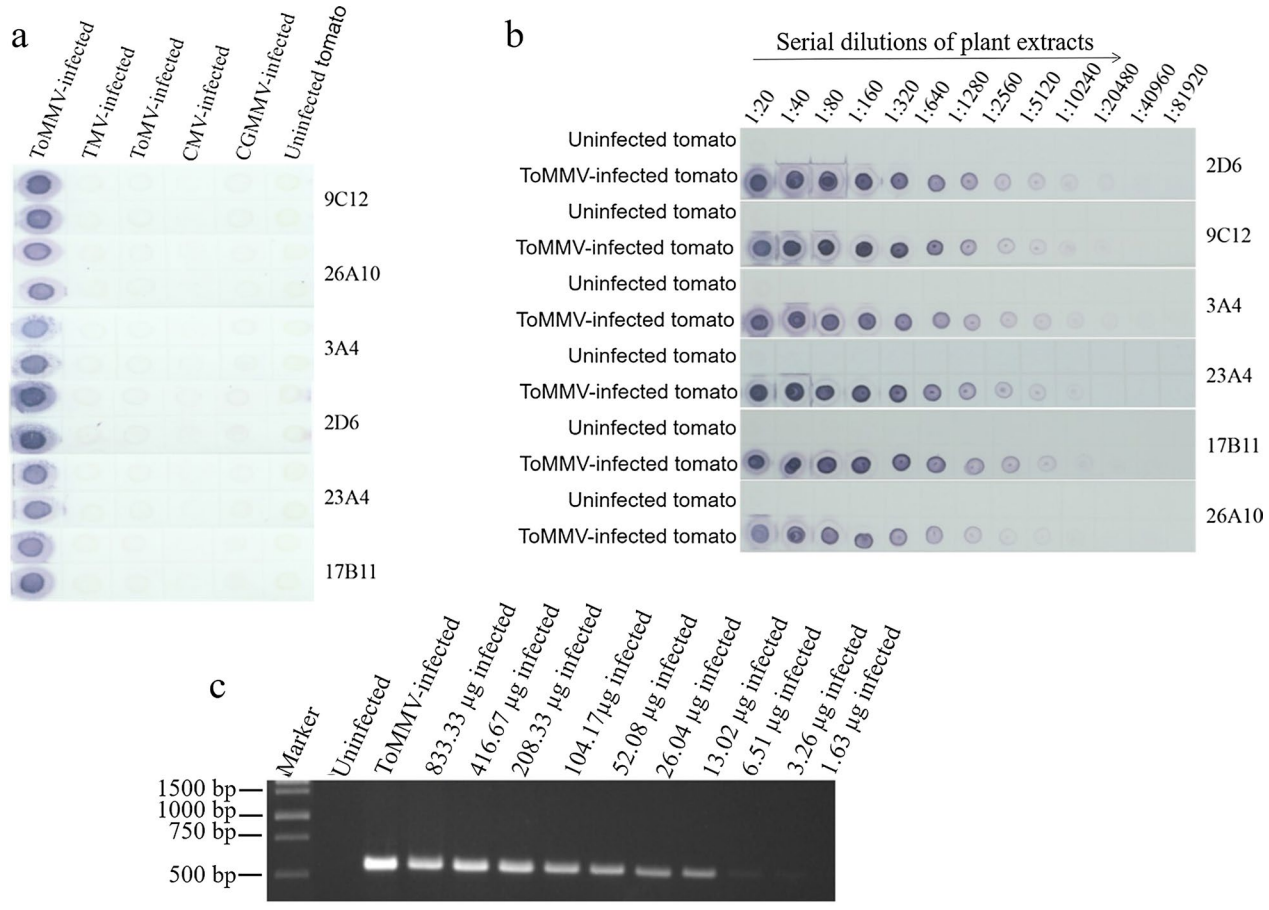

Fig. 4 Specificity and sensitivity analyses of the developed dot-ELISAs and conventional RT-PCR. **a** Specificity evaluation of the developed dot-ELISAs. Extracts were prepared from ToMMV-, ToMV-, TMV-, CMV- or CGMMV-infected plant tissues. The extract from uninfected tomato samples was used as a negative control. For each MAb, two dots on the same membrane were two biological repeats. The light to dark purple color indicates a positive reaction. **b** Sensitivity evaluation of the developed dot-ELISAs. The extracts from ToMMV-infected and uninfected tomato plants were serially diluted from 1:20 to 1:81,920 (w/v) in PBS (pH 7.4). The light to dark purple color indicates a positive reaction. **c** Specificity and sensitivity evaluations of the one-step RT-PCR method. Total RNA (30 μL) purified from 100 mg of ToMMV-infected tomato plant tissues was serially two-fold diluted from 1:4 to 1:2048 (w/v) in DNase/RNase free ddH_2O, and 1 μL of each RNA dilution was used to determine the sensitivity

or CGMMV-infected plants, cut transversely, and then the cut surface of each stem was printed on a nitrocellulose membrane. The stems from uninfected tomato plants were used as negative controls. The membrane was probed with individual MAbs followed by AP-conjugated goat anti-mouse IgG second antibody. The results showed that the prints from ToMMV-infected tomato stems presented a dark purple color, while prints from other plant stems remained colorless or presented a light green color (Fig. 5), indicating that no cross-reactions occurred between the MAbs and ToMV-, TMV-, CMV- or CGMMV-infected plants, or uninfected tomato plants.

Detection of ToMMV infection in field-collected tomato plant samples

To determine the usefulness of three newly developed serological methods for the detection of ToMMV infection, we collected 38 tomato plants showing virus-like symptoms from fields in Yunnan and Hainan provinces during 2018–2019, and tested them for ToMMV infection using ACP-ELISA, dot-ELISA and tissue print-ELISA as described above. It was showed that 16 samples of them were infected with ToMMV (Fig. 6a–c). To further validate the result, we analyzed these 38 samples through RT-PCR using ToMMV *RdRp*-specific primers. The results demonstrated that the 16 ToMMV-positive samples determined through serological detections also gave a specific amplification product of ~ 600 bp, but the remaining 22 samples along with the uninfected negative control didn't give this target product (Fig. 6d). Sequencing of the 16 PCR products confirmed that they are fragments of the ToMMV *RdRp* gene.

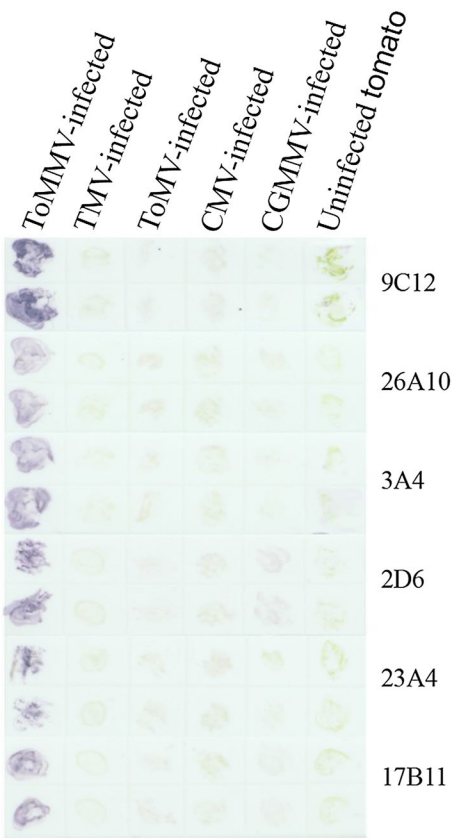

Fig. 5 Specificity analyses of tissue print-ELISAs. Columns 1 to 6 were the prints from ToMMV-, ToMV-, TMV-, CMV- or CGMMV-infected plant stems, respectively. Prints from uninfected tomato plants were used as negative controls. The upper and lower prints in each treatment were from two different plant samples. MAbs used are indicated on the right side of the blots. Purple color indicates a positive reaction

Discussion

ToMMV can survive in plant debris or on seed surface for a long period of time, consequently, the contaminated seeds and infected plant debris have always been the main sources of the virus, by which ToMMV is spread over long distances. In solanaceous crop fields, ToMMV can be further spread to adjacent plants through mechanical contacts, including pruning, plant staking, grafting and fruit harvesting (Li et al. 2017; Chen et al. 2018; Zhan et al. 2018). In recent years, ToMMV has been reported to cause serious damages to tomato, eggplant and pepper crops in many countries. To prevent and reduce ToMMV-caused losses, new reliable, high-throughput and low-cost virus detection methods are urgently needed. Current ToMMV detection methods mainly include symptom observation, RT-PCR and electronic microscopy observation (Padmanabhan et al. 2015; Sui et al. 2017; Zhan et al. 2018). Since many tobamoviruses, including TMV and ToMV, cause symptoms similar to that of ToMMV, symptom observation cannot be used to identify these causal viruses in infected plants. Detection of specific virus infection in plant through RT-PCR is reliable. This method, however, is time-consuming and needs expensive equipment. Thus, it is not suitable for large-scale field surveys (Abraham et al. 2012). Electronic microscopy observation needs not only expensive equipment but also well-trained technician. In addition, it cannot distinguish viruses with similar particle morphologies. Serological methods are fast, simple-to-use and specific for virus detection, and therefore suitable for large-scale testing (Wu et al. 2013; Zhang et al. 2018; Guo et al. 2020). It is commonly accepted that the reliability of a serological test depends largely on the sensitivity and specificity of the antibodies used. Therefore, the preparation of high-quality antibodies with high sensitivity and specificity is crucial for the establishment of reliable serological methods for plant virus detection.

To date, no ToMMV-specific antibody or serological detection method has been reported (Zhan et al. 2018).

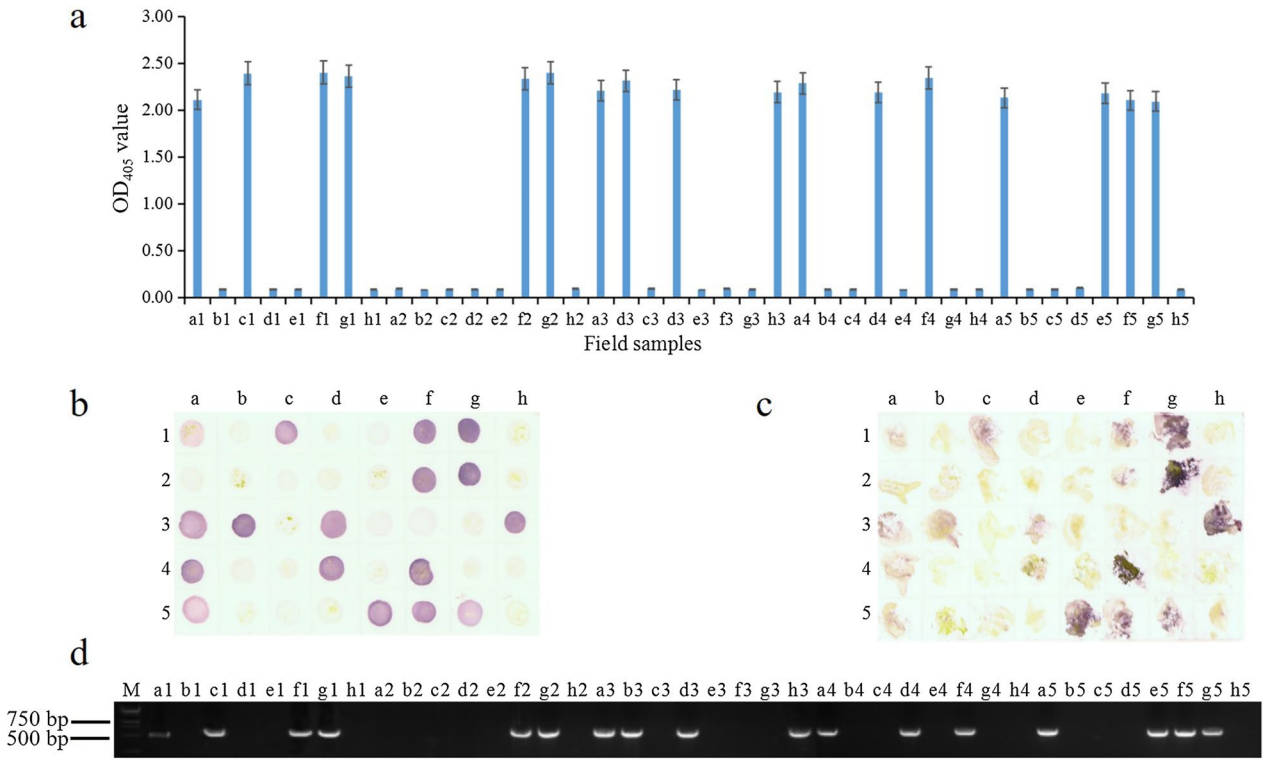

Fig. 6 Detection of ToMMV infection in field-collected tomato samples through ACP-ELISA (**a**), dot-ELISA (**b**), tissue print-ELISA (**c**) and RT-PCR (**d**). Samples were prepared from 38 field-collected tomato plants and named as a1 to a5, b1 to b5, c1 to c5, d1 to d5, e1 to e5, f1 to f5, g1 to g4 and h1 to h4. Samples g5 and h5 represent a ToMMV-infected and an uninfected tomato plant, and were used as positive and negative controls, respectively. MAb 2D6 was used as the detection antibody in ACP-ELISA, dot-ELISA and tissue print-ELISA assays. The primer pair used in RT-PCR is ToMMV *RdRp*-specific and can amplify a fragment of ~ 600 bp

Due to the huge threat caused by ToMMV to solanaceous crops, we aimed at developing reliable large-scale serological detection methods for this virus in this study. We purified ToMMV from infected tomato leaf tissues and used it as an immunogen to produce six anti-ToMMV MAbs through the murine hybridoma technology. Our Western blot analysis showed that these six MAbs can be used to detect ToMMV infection specifically and sensitively. Using these MAbs as the detection antibodies, we have now developed ACP-ELISAs, dot-ELISAs and tissue print-ELISAs for ToMMV detection. The test results showed that these newly developed serological detection methods can be used to reliably detect ToMMV infection in tomato plants without cross-reaction to TMV, ToMV, CGMMV and CMV (Figs. 3a, 4a, 5). Our serial dilution test results indicate that ACP-ELISA and dot-ELISA can be used to detect ToMMV infection in leaf extracts diluted up to 1:81,920 and 1:20,480, respectively. Surprisingly, the detection limit of the developed dot-ELISA is much higher (26 times) than that of RT-PCR. Detection of ToMMV infection in field-collected tomato samples showed that the three developed serological methods are as reliable as conventional RT-PCR. Considering that these three serological methods are reliable, fast and easy-to-use, we recommend them for large-scale detection of ToMMV infection in field surveys. These methods can also be used in the corresponding resistance breeding projects. In addition, the six MAbs can be used to determine the accumulation of ToMMV in plants during studies of host plant-ToMMV interactions.

Conclusions

In this study, we prepared six MAbs that are specific and sensitive to ToMMV through the hybridoma technology, and developed three serological methods (ACP-ELISAs, dot-ELISAs and tissue print-ELISAs) for the detection of ToMMV using the produced MAbs. All these three serological methods can be used to reliably and sensitively detect ToMMV infection in plants without cross-reaction to TMV, ToMV, CGMMV and CMV. Furthermore, the serological methods described in this study are suitable for large-scale field detection of ToMMV and provide crucial information for the disease management.

Methods

Virus source and plant sample collection

Uninfected and ToMV-, ToMMV-, TMV-, CMV- or CGMMV-infected plants were maintained separately in insect-proof greenhouse. ToMMV infection was confirmed by RT-PCR in tomato plants, which were used for subsequent virion purification. ToMMV virions were purified from the infected tomato plant tissues using the method reported previously (Zhou et al. 1994). During growth seasons in 2018 and 2019, 38 samples were collected from tomato plants with virus-like symptoms in fields in Hainan and Yunnan provinces, China.

MAb preparation

Purified ToMMV virions were used to immunize BALB/c female mice as described previously (He et al. 2020). Hybridoma cells secreting anti-ToMMV MAbs were selected and preparations of ascitic fluids containing MAbs were carried out as described by Huang et al. (2019). Crude extracts from ToMMV-inoculated plant tissues were used as the coating antigen in the indirect-enzyme-linked immunosorbent assay (in-ELISA) to determine titers of the resulting ascitic fluids. The specificities and sensitivities of MAbs were determined through Western blot analysis and antigen-coated-plate (ACP)-ELISA as described previously (Wu et al. 2013).

Detection of ToMMV using ACP-ELISA

In ACP-ELISA, serially diluted antibody solutions were used to determine the working dilutions of ToMMV-specific MAbs and alkaline phosphatase (AP)-conjugated goat anti-mouse IgG second antibody (Sigma-Aldrich) as described by Shang et al. (2011). The ACP-ELISA was carried out as described previously (Liu et al. 2017). Briefly, plant tissues were individually homogenized in 0.05 mol/L sodium bicarbonate buffer (pH 9.6) at a ratio of 0.1 g tissue per 3 mL buffer. The crude extracts were centrifuged at 5 000 *g* for 3 min, and the supernatant of each sample was used for virus detection. Wells of microtiter plates were loaded with 100 µL of extracts from ToMMV-, ToMV-, TMV-, CMV- or CGMMV-infected plants, and the plates were incubated overnight at 4 °C, and extracts from the confirmed ToMMV-infected and uninfected tomato plants were used as positive and negative controls, respectively. After three rinses with PBST (0.01 mol/L PBS with 0.05% Tween-20, pH 7.4), each well was incubated with 250 µL blocking buffer (PBS with 3% dried skimmed milk) for 30 min at 37 °C. Diluted ToMMV MAb solutions (100 µL) were added into each well, and the plates were incubated for 1 h at room temperature (RT). After three rinses with PBST, 100 µL diluted AP-conjugated goat anti-mouse IgG second antibody solution was added to each well, and the plates were incubated for 1 h at RT. After four rinses with PBST, 100 µL ρ-nitrophenyl phosphate substrate solution was added into each well, and the plates were incubated for 30 min in the dark at RT. The optical density value at 405 nm (OD_{405}) of each well was measured with a Microplate Reader Model 680 (Bio-Rad, Hercules, USA).

Dot-ELISA and tissue print-ELISA

Dot-ELISA was carried out as described previously with specific modifications (Chen et al. 2017). Approximately 100 mg of tomato plant tissues was homogenized in 3 mL 0.01 mol/L PBS (pH 7.4). The homogenate was centrifuged at 5000 *g* for 3 min at 4 °C, and the resulting supernatant was used for virus detection. Plant extracts from the confirmed ToMMV-infected and uninfected tomato plants were used as positive and negative controls, respectively. About 2.5 µL supernatant was taken from each sample and blotted onto a nitrocellulose membrane followed by air-drying at 37 °C for 5 min. The blotted nitrocellulose membrane was incubated in PBST solution containing 5% dried skimmed milk for 30 min followed by incubation in a diluted ToMMV MAb solution for 1 h. The membrane was then incubated for 1 h in a diluted AP-conjugated goat anti-mouse IgG second antibody solution. After four rinses in PBST, the detection signal was visualized by addition of a nitro-blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate solution.

Tissue print-ELISA was performed as described previously (Shang et al. 2011). Stems were harvested from ToMMV-, ToMV-, TMV-, CMV- or CGMMV-infected plants, with stems from uninfected plants as negative controls. The stems were cut transversely with a blade and the cut surface of each stem was immediately pressed for 3–5 s against a nitrocellulose membrane. The following detection steps were the same as that described for dot-ELISA above.

RT-PCR and sequence analysis

According to the ToMMV *RdRp* gene sequences deposited in the GenBank database, we synthesized a forward primer (5'-GAAAAGGGCGGTCTAATTTCC-3') and a reverse primer (5'-ACACTTCCTTTAATTTTCGTCCT-3'), which were used for amplification of a conserved fragment from the ToMMV genome (nucleotide position 5595–6228, GenBank accession number MH381817.1). Using a one-step RT-PCR kit (Toyobo Life Science, Shanghai, China), the sensitivity of RT-PCR for ToMMV detection was analyzed. In addition, RT-PCR was used to detect ToMMV infection in field-collected samples and the resulting products were sequenced and used to

search GenBank database for validation. RT-PCR was then used to confirm the result of serological detection.

Abbreviations

ACP-ELISA: Antigen-coated-plate enzyme-linked immunosorbent assay; CGMMV: Cucumber green mottle mosaic virus; CMV: Cucumber mosaic virus; CP: Coat protein; DAS-ELISA: Double antibody sandwich enzyme-linked immunosorbent assay; dot-ELISA: Dot enzyme-linked immunosorbent assay; MAb: Monoclonal antibody; ORF: Open reading frame; PBS: Phosphate buffer; RT-PCR: Reverse transcript-polymerase chain reaction; Tissue print-ELISA: Tissue print enzyme-linked immunosorbent assay; ToMMV: Tomato mottle mosaic virus; ToMV: Tomato mosaic virus; TMV: Tobacco mosaic virus.

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Authors' contributions

XL and LG prepared the MAbs and drafted the manuscript. MG and DQ conducted dot-ELISA and tissue print-ELISA experiments. MG and DQ collected the samples. XZ edited the manuscript. FL and JW conceived the study and revised the manuscript. JW proof-read and finalized the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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