

# Development of polyclonal antisera against movement proteins of the three poleroviruses infecting cucurbits and their serological relationships

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## Research

**Keywords:** Cucurbit aphid-borne yellows virus, Melon aphid-borne yellows virus, Suakwa aphid-borne yellows virus, movement protein, prokaryotic expression, antiserum preparation, virus detection

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# Abstract

**Background:** *Cucurbit aphid-borne yellows virus* (CABYV), *Melon aphid-borne yellows virus* (MABYV) and *Suakwa aphid-borne yellows virus* (SABYV) are three critical viruses infecting cucurbit crops. The preparation of specific antiserum against the virus is crucial for both the detection of virus and understanding the functions of the related genes. However, there is no report about detecting the three viruses using antisera against movement proteins (MP).

**Methods:** In this study, we constructed prokaryotic expression vectors of the three viral movement proteins and transferred them into *Escherichia coli* strain Rosetta to purify the fusion proteins. Then the polyclonal antisera were obtained by immunizing New Zealand white rabbits. Western blotting was used to demonstrate the applicability of the three antisera.

**Results:** We discovered that the titer of antiserum against MP CABYV reached to 1: 512000, and the titers of antisera against MP MABYV and MP SABYV reached to 1:256000. The optimized working concentration range for the three antisera was from 1:10000 to 1:64000. Both antisera against MP CABYV and MP MABYV could only react with the corresponding MP. The antiserum against MP SABYV not only had the strongest reaction with its MP but also could react with MP CABYV and MP MABYV at relative weaker levels and all the three antisera had no serological reactions with other polioviruses tested. Furthermore, our results showed that the three antisera could specifically detect movement proteins both in *Nicotiana benthamiana* and cucumber leaves.

**Conclusions:** We have established a sensitive system for detecting three polioviruses infecting cucurbits by antisera against movement proteins, providing a material foundation for the future research on both the serological detection of viruses and the interaction mechanisms between the virus and host plants.

## Background

Cucurbitaceae is one of the most important fruits and vegetable crops in the world. Various plant viruses infecting cucurbits cause significant economic losses, which has become a limiting factor for the production of cucurbits [1–3]. Cucurbit aphid-borne yellows virus (CABYV), Melon aphid-borne yellows virus, (MABYV) and Suakwa aphid-borne yellows virus (SABYV) are important viruses infecting cucurbits, belonging to the genus poliovirus, the family Luteoviridae [4–6]. CABYV was first reported to infect cucurbit crops in France in 1992 and later found in many other countries including China [4, 7–14]. Interestingly, CABYV can also infect passion fruit and pepper naturally, which causes a large area of yellowing symptoms in fields [15–17]. MABYV and SABYV were first reported in China in 2008 and 2009, respectively [5, 6]. MABYV is widely distributed in many provinces in China and Thailand; however, SABYV has been only detected in coastal provinces such as Fujian and Guangdong in southern China [2, 6]. Subsequently, SABYV was reported in Southeast Asian countries such as Thailand, the Philippines, and East Timor [18–20]. All the three viruses are limited to the phloem tissues of the host plant and are transmitted by aphids in a persistent circulative and non-proliferative manner. They are highly specific in

vectors and cannot be transmitted mechanically [21]. Symptoms such as yellowing and thickening of old leaves could cause severe yield reductions. However, all three viruses have no significant effect on the quality and shape of fruits [4–7].

The virion of polerovirus including CABYV, MABYV and SABYV is a ball-shaped icosahedron with diameters of 25–30 nm, which encapsulate genome RNA. The virions are relatively stable and are not sensitive to chloroform and non-ionic detergents, but can be destroyed under high salt conditions with long time [22]. The genome consists of a single positive-stranded RNA approximately 5.7 kb in length, containing seven open reading frames (ORFs). The first three ORFs of them are expressed by genomic RNA and the others are expressed by subgenomic RNA [23]. Specifically, the P0 protein encoded by ORF0 is a classic RNA silencing suppressor, which can enhance the pathogenicity of viruses and can promote the accumulation of virus through interacted with host plants genes [24–29]. The P1 protein encoded by ORF1 serves as protease, helicase, and VPg. A P1-P2 fusion protein is encoded through frameshift and has a replicase activity [22]. The intergenic-non-coding region (intergenic-NCR) approximately 80 nt in length locates between ORF2 and ORF3a. The P3a protein without AUG initiation encoded by ORF3a is indispensable for the long-distance movement of the virus [30–31]. The coat protein is encoded by ORF3, participating in the packaging, long-distance movement, and aphids transmission of the virus [32]. ORF4 encodes MP through leaky scanning, which could locate in plasmodesmata, mitochondria, and chloroplast to regulate much infection process including replication, cell to cell movement, and long-distance movement [33–34]. The read-through protein encoded by ORF5 is closely related to aphids transmission, virion assembly, long-distance movement, and phloem limited and so on [35–38].

Serological methods, as one of the essential methods for virus detection, are widely used due to its simple preparation, strong specificity, and high sensitivity. In the current research, RT-PCR is mainly used for the detection of CABYV, MABYV, and SABYV [18, 39]. In regards to the serological detection, only antiserum against CABYV prepared by purified virion was once used in Western blotting or ELISA [4]. Some recent studies showed that polyclonal antiserum against MP can detect the accumulation of virus from the family Luteoviridae [40–43]. However, there are no reports on either preparing polyclonal antiserum against MP of the three viruses or the serological relationships among them. In our work, in order to obtain polyclonal antisera, we used His-MBP-MP purified fusion protein by prokaryotic expression system to immunize New Zealand rabbits. Western blotting showed that all the three viruses were detected by these antisera specifically. We confirm the serological relationship among them and provide materials for future research on both the serological detection of viruses and the interactions between the virus and host plants.

## Results

# Prokaryotic expression and purification of MP fusion proteins and development of antisera

MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup> genes were constructed into the pDB-His-MBP vector, which was transformed into Escherichia coli strain Rosetta to be expressed and purified. Then SDS-PAGE indicated that all the three recombinant proteins showed specific bands corresponding to 65 KDa, which is as predicted. Based on the results of SDS-PAGE, the purification is successful. His-MBP-CABYV-MP specific fusion protein was eluted at concentrations of 200 and 500 mM imidazole (Fig. 1A), His-MBP-MABYV-MP specific fusion protein was eluted at concentrations of 100 and 200 mM imidazole (Fig. 1B) and His-MBP-SABYV-MP specific fusion protein was eluted at concentrations of 80, 100 and 200 mM imidazole (Fig. 1C), respectively. We sent 3 mg of the concentrated proteins respectively to Beijing Protein Innovation Co.Ltd. (BPI) to immunize New Zealand white rabbits and approximately 30 mL of each polyclonal antiserum was successfully obtained.

## Titer analysis of the three antisera

We constructed MP<sup>CABYV</sup>, MP<sup>MABYV</sup>, and MP<sup>SABYV</sup> into the pMDC32 vector to express target proteins. Then we transiently expressed all the three MPs in *N. benthamiana* via agro-infiltration and the pMDC32 empty vector served as the negative control. Leaves were collected at three dpi to extract total proteins for western blotting, which showed that all the antisera could detect the corresponding MP respectively. Furthermore, antiserum against MP<sup>CABYV</sup> still could detect the specific band when the antiserum was diluted to 512000 times (Fig. 2A). However, both antiserum against MP<sup>MABYV</sup> and antiserum against MP<sup>SABYV</sup> can detect the specific bands when these two antisera were diluted to 256000 times (Fig. 2B and 2C). In summary, the titer of antiserum against MP<sup>CABYV</sup> reached to 1:512000 and both the titers of the other two antisera reach to 1:256000. From the perspective of color appearance and economics, the optimal working concentration range of the three antisera was from 1: 10000 to 1: 64000.

## Sensitivity analysis of the three antisera

To analyze the sensitivity of the three antisera, *N. benthamiana* leaves expressing MP<sup>CABYV</sup>, MP<sup>MABYV</sup>, and MP<sup>SABYV</sup>; the total proteins were extracted and diluted in equal proportion. Western blotting showed that the antiserum against MP<sup>CABYV</sup> diluted at 1:1000 could detect sample diluted to 128 times. Similarly, sample diluted to 64 times could be detected by the antiserum against MP<sup>MABYV</sup> and sample diluted to 40 times could be detected by the antiserum against MP<sup>SABYV</sup> used at the same ratio. Furthermore, samples diluted to 80 times, 16 times, and 32 times could be detected by the antisera against MP<sup>CABYV</sup>, MP<sup>MABYV</sup>, and MP<sup>SABYV</sup> diluted at 1:10000 respectively. When we diluted antisera at 1:20000, antiserum against MP<sup>CABYV</sup> showed a higher sensitivity and samples diluted to 64 times still could be detected. However, under the same conditions, the other two antisera showed a lower sensitivity and samples diluted to 8 times could be detected (Fig. 3).

## Specificity analysis of the three antisera

To analyze the specificity of the three antisera, we transiently expressed various MPs including MP<sup>CABYV</sup>, MP<sup>MABYV</sup>, MP<sup>SABYV</sup>, 3Flag-MP<sup>BrYV</sup> (Brassica yellow virus), 3Flag-MP<sup>PLRV</sup> (Potato leafroll virus) and 3Flag-MP<sup>ScYLV</sup> (Sugarcane yellow leaf virus) in *N. benthamiana* via agro-infiltration. The pMDC32 empty vector served as a negative control. Leaves were collected at 3 dpi to extract total proteins for western blotting. The antisera were diluted at 1:1000, 1:10000 and 1:20000 respectively. Both antisera against MP<sup>CABYV</sup> and MP<sup>MABYV</sup> could only detect the corresponding MP no matter how antisera were diluted, showing a high specificity (Fig. 4A and 4B). Interestingly, although antiserum against MP<sup>SABYV</sup> had the strongest reaction with MP<sup>SABYV</sup> at various dilution concentrations, MP<sup>CABYV</sup> and MP<sup>MABYV</sup> were still detected at weaker levels. This means that antiserum against MP<sup>SABYV</sup> could detect three different poleroviruses infecting cucurbits specifically (Fig. 4C). Furthermore, all the three antisera had no serological cross-reactivity with other MPs from the same genus, including MP<sup>PLRV</sup>, MP<sup>BrYV</sup> and MP<sup>ScYLV</sup>.

## Application of the antisera in the virus detection

We inoculated CABYV and MABYV infectious clones into *N. benthamiana* via agro-infiltration. Then we collected inoculated leaves at 3 dpi to extract total protein for western blotting. Detection results showed that both antisera specifically detected the CABYV and MABYV in *N. benthamiana* leaves, which is corresponding to the RT-PCR detection results (Fig. 5A and 5B). The results showed that our antisera were suitable for the detection of viruses in *N. benthamiana*, the most widely used model plant in plant virology.

To test whether the antisera can detect MP in the natural host such as cucumber infected with a virus, we simulated the situations in which cucumber was infected with the virus, by mixing the diluted total protein-containing transient expressed MPs in *N. benthamiana* leaves with the extract of healthy cucumber leaves. Western blotting showed that protein samples diluted to 256 times, 64 times and 128 times could be specifically detected by the antisera against MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup>, respectively (Fig. 5C). Total protein from healthy cucumber leaves had no serological reaction with the three antisera and no interference with the specific serological responses of the three antisera to their corresponding MP protein.

## Discussion

To some extent, the successful infection of the virus depends on the efficient movement in hosts. Blocking the movement of viruses provides strategies for host plants to resist viruses. Therefore, it is important to study how MP participates in the movement of the virus in hosts. MP plays an essential role in virus infection, which not only promotes the intercellular movement and systemic movement of the virus but also serves as a critical pathogenic factor to cause purple symptoms in the infected leaves [44]. Thus, we establish a specific detection system by developed antisera against three different MPs, which is indispensable to study the occurrence and distribution of viruses and the function of MP.

Among the three developed antisera, antisera against MP<sup>CABYV</sup> and MP<sup>MABYV</sup> have shown higher specificities as they specifically reacted with their targeted proteins, whereas antiserum against MP<sup>SABYV</sup> could react with all three viruses in serology at the same time. Interestingly, we can distinguish three different viruses by the strength of the serological response, and the strongest response was MP<sup>SABYV</sup>, followed by MP<sup>MABYV</sup> and the weakest response was MP<sup>CABYV</sup>. MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup> share higher homology amongst them. At the nucleotide level, MP<sup>SABYV</sup> shares 79.7% identity with MP<sup>CABYV</sup> and 88.5% identity with MP<sup>MABYV</sup>. At the amino acid level, MP<sup>SABYV</sup> only shares 65.4% identity with MP<sup>CABYV</sup> and 75.1% identity with MP<sup>MABYV</sup>, which is positively correlated with the strength of the serological response.

Cucurbit crops such as cucumber, watermelon, melon, and pumpkin are the natural hosts of CABYV, MABYV and SABYV. The antisera we have developed are not only available for MP detection in the model plant *N. benthamiana*, but also suitable in natural hosts. The simulating detection suggested that total protein from cucumber did not interfere with the specific reaction between antisera and MPs. Meanwhile, CABYV could be detected in the natural host passion fruit by the developed antiserum against MP<sup>CABYV</sup>, which provides new evidence for the application of the antiserum in fields [17]. Our work showed that the three antisera have a wide range of applications, although the detection on cucurbits needs further verification.

The antiserum developed based on the MP of Barley yellow dwarf virus (BYDV)-PAV Qinghai isolate not only reacted with PAV105 isolate sharing 79.3% identity in amino acid, but also had strong serological cross-reactivity with the other BYDV MPs sharing 62.7%-83.3% identity in amino acid [43]. Among poleroviruses, Luffa aphid-borne yellows virus (LABYV), Pepo aphid-borne yellows virus (PABYV), and Cucumber aphid-borne yellows virus (CuABYV) also infect cucurbit crops [45]. In 2019, Zucchini aphid-borne yellows virus (ZABYV) was reported as a new virus species in China [46]. The MPs of these viruses have 23.08% -75.92% identity with the MPs of CABYV, MABYV, and SABYV in amino acid (Table 1). PABYV MP showed the highest homology, and LABYV MP showed the lowest homology among them. However, further experiments are indispensable to investigate the serological relationship between MPs of these viruses and the three antisera we developed.

Table 1  
Comparison of amino acid sequence identities (%) of movement protein between CABYV, MABYV, SABYV and other poleroviruses infecting cucurbits

Poleroviruses	Accession Number	CABYV	MABYV	SABYV
LABYV	NC_027703	24.27	26.83	23.08
PABYV	NC_030225	75.92	70..31	71.88
CuABYV	FJ460218	59.28	59.28	60.31
ZABYV	MK050791	56.48	55.44	55.96

## Conclusions

Diseases caused by CABYV, MABYV and SABYV are important viral diseases in the cucurbit crops. Development of specific antiserum is essential for virus detection and understanding the functions of the related genes. In our study, we purified three MPs by the prokaryotic expression system and developed polyclonal antisera with high titer, sensitivity, and specificity, although antiserum against MP<sup>SABYV</sup> had a relatively weaker reaction with MP<sup>CABYV</sup> and MP<sup>MABYV</sup>, which can be successfully used for virus detection and exploring the mechanisms of viral replication, movement and interaction with host plants.

## Materials And Methods

### Plant materials and grown conditions

The seeds of *Nicotiana benthamiana* were kindly donated by Professor David Baulcombe (Department of Plant Sciences, University of Cambridge, U.K), which were propagated and preserved in this laboratory. *Nicotiana benthamiana* was grown at the conditions of  $24 \pm 1^\circ\text{C}$ , 16/8 hours light / dark cycle and forty percent humidity.

### The construction of vectors

The full-length of MP<sup>CABYV</sup>, MP<sup>MABYV</sup>, and MP<sup>SABYV</sup> were amplified by PCR using forward and reverse primers (Table 2). The PCR products were recycled by 1.0% agarose gel electrophoresis and linked into the vector pDB.His.MBP (DNASU Plasmid Repository, Arizona, USA) digested with restriction enzymes NdeI and XhoI at  $37^\circ\text{C}$  for more than 4 hours. We transformed the ligation products into *E.coli* strain MC1022 to obtain the positive clones and extracted plasmids. Sequencing analysis verified the correctness of plasmids. pMDC32-MP<sup>CABYV</sup>, pMDC32-MP<sup>MABYV</sup> and pMDC32-MP<sup>SABYV</sup> were constructed using the same methods [47].

Table 2  
Specific primers used for vector construction

Vector Name	Primer Name	primer sequence
PDB.His.MBP	CABYVMPNdel	AACCTTTACTTCCAGGGCCATATGCAGGGAGGCGGAGGCCGA
	CABYVMPXhol	GGTGGTGGTGGTGGTCTCGAGTCCTATTTTCGGGTTTTGGACC
	MABYVMPNdel	AACCTTTACTTCCAGGGCCATATGGCATGGGAAGGAGGAGA
	MABYVMPXhol	GGTGGTGGTGGTGGTCTCGAGTCTACCTATTTTCGGGTTCTGG
	SABYVMPNdel	AACCTTTACTTCCAGGGCCATATGGCATGGGAAGAAGGAGA
	SABYVMPXhol	GGTGGTGGTGGTGGTCTCGAGTCTACCTATTTTCGGGTTCTGG
pMDC32	CABYVMPKpnl	GGGGTACCATGCAGGGAGGCGGAGGCCGA
	CABYVMPSpel	GGACTAGTCCTATTTTCGGGTTTTGGACC
	MABYVMPKpnl	GGGGTACCATGGCATGGGAAGGAGGAGA
	MABYVMPSpel	GGACTAGTCTACCTATTTTCGGGTTCTGG
	SABYVMPKpnl	GGGGTACCATGGCATGGGAAGAAGGAGA
	SABYVMPSpel	GGACTAGTCTACCTATTTTCGGGTTCTGG

## Agrobacterium-mediated transformation

The liquid nitrogen freezing and thawing method were used to transform the transient expression vector plasmids into Agrobacterium. 3–5 $\mu$ L plasmids were added into 100 $\mu$ L Agrobacterium competent cells GV3101, which was on the ice for 30 minutes and frozen in liquid nitrogen for 1 minute. Then it was melted at 37°C for 5 min, and then 400 $\mu$ L LB liquid medium was added. After 4 hours of oscillation resuscitation at 28°C, it was spread on the corresponding resistant LB plate. The transformed agrobacterium competent cells were grown at 28°C for 48 h.

## The purification of recombinant proteins and the development of polyclonal antisera

We transformed three prokaryotic expression vectors containing MP into E.coli strain Rosseta to obtain the positive colonies. Positive colonies were cultured in 10 ml LB liquid medium containing kanamycin (50 mg/ml) and chloramphenicol (34 mg/ml) at 37 °C and 220 rpm overnight for oscillating culture. All cultured bacteria were added to 1L LB liquid medium containing kanamycin and chloramphenicol at 37°C, and 220 rpm for oscillating culture for 4–6 h in order to OD<sub>600</sub> ranged from 0.6 ~ 0.8. Then the transformed bacteria were induced by shaking at 18°C and 180 rpm for 18 h and Isopropyl- $\beta$ -D-thiogalactoside (IPTG; Sigma-Aldrich, St. Louis, MO, USA) was added at a final concentration of 0.1 mM. We collected the bacteria by centrifugation at 5000 rpm for 6 minutes. After Ultrasonic crushing, we

collected supernatant by centrifugation at 16000 rpm for 40 minutes. The supernatant was added to a Ni-affinity column (Qiagen, Hilden, Germany) and the proteins were eluted with imidazole eluent of different concentrations (20 mM, 40 mM, 60 mM, 80 mM, 100 mM, 200 mM, and 500 mM). Each elution was subjected to SDS-PAGE for detecting whether MP was expressed and elution containing MP was concentrated to obtain purified fusion proteins.

The three fusion proteins then were immunized New Zealand white rabbits. When the immunization was completed, positive blood was taken from the carotid artery of New Zealand white rabbits, after centrifuged twice at 5000 rpm for 10 min, 30 ml of polyclonal antisera against MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup> were obtained respectively. (Beijing Protein Innovation Co.Ltd conducted the rabbit immunization).

## Western blotting assay

Proteins were separated by SDS-PAGE and then transferred to the nitrocellulose membrane (GE Healthcare, Buckinghamshire, UK) by electrotransfer (200 mA, 100 min). The NC membrane was blocked in 1 × TBST buffer (20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, pH = 8.0) containing 5% milk at 37°C for 1 h, followed by incubated with polyclonal antisera against MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup> at 37°C for 1 h. Subsequently, We washed the membrane with 1 × TBST for three times and incubated the NC membrane with AP-labeled goat anti-rabbit IgG secondary antibody (Easybio, China) diluted to 1:20000 at 37°C for 1 h. After washing the membrane with 1 × TBST for three times, we incubated the NC membrane with the buffer containing NBT (330 µg.mL<sup>-1</sup>) and BCIP (165 µg.mL<sup>-1</sup>) to show corresponding bands.

## Titer determination of the three antisera

pMDC32-MP<sup>CABYV</sup>, pMDC32-MP<sup>MABYV</sup> and pMDC32-MP<sup>SABYV</sup> (OD<sub>600</sub> = 0.5) was co-expressed with P19 (OD<sub>600</sub> = 0.2) in *N. benthamiana*, and the pMDC32 empty vector served as the negative control. Leaves were collected at three dpi for extracting total protein. All the three antisera were equally diluted followed by 1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000, 1:64000, 1:128000, 1:256000 and 1:512000 in Western blotting experiments to determine its titer and optimal working concentration.

## Sensitivity analysis of the three antisera

pMDC32-MP<sup>CABYV</sup>, pMDC32-MP<sup>MABYV</sup> and pMDC32-MP<sup>SABYV</sup> (OD<sub>600</sub> = 0.5) were co-expressed with P19 (OD<sub>600</sub> = 0.2) in *N. benthamiana* and the pMDC32 empty vector served as the negative control. Leaves were collected at 3 dpi for extracting total protein. We weighed 0.1 g of *N. benthamiana* leaves and added 300 µL 2xSDS buffer to prepare protein samples. The samples were diluted by two gradients (1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:40, 1:80, 1:160, 1:320, 1:640) in Western blotting experiments. Meanwhile, all three antisera were diluted to 1:1000, 1:10000, and 1:20000 respectively.

## Specificity analysis of the three antisera

MP<sup>CABYV</sup>, MP<sup>MABYV</sup>, MP<sup>SABYV</sup>, 3Flag-MP<sup>BrYV</sup>, 3Flag-MP<sup>PLRV</sup> and 3Flag-MP<sup>ScYLV</sup> (OD<sub>600</sub> = 0.5) was co-expressed with P19 (OD<sub>600</sub> = 0.2) in *N. benthamiana*, and the pMDC32 empty vector served as the negative control. Leaves were collected at 3 dpi for extracting total protein. We performed western blotting to detect specificity of the antisera. The three antisera were all diluted at the ratio of 1:1000, 1:10000 and 1:20000 respectively.

Detection of *N. benthamiana* inoculated with full-length infectious clones

We inoculated *N. benthamiana* with the full-length infectious clone of CABYV and MABYV [48–49]. Transient expression vectors pMDC32-MP<sup>CABYV</sup> and pMDC32-MP<sup>MABYV</sup> served as the positive control and the pCass-RZ empty vector served as the negative control. Western blotting was performed using antisera at 1:10000, which was compared with the RT-PCR detection of the same sample.

## Simulation detection of natural host plants with infected virus

Total proteins from *N. benthamiana* leaves expressing MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup> were extracted followed by the same dilution ratio as healthy cucumber leaves (1:4). Then we mixed them with equal volume, which was equally diluted by 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512. *N. benthamiana* leaves expressing the pMDC32 empty vector, and healthy cucumber leaves served as the negative controls. While *N. benthamiana* leaves expressing MP<sup>CABYV</sup>, MP<sup>MABYV</sup> and MP<sup>SABYV</sup> served as the positive control respectively. Then we conducted Western blotting using antisera at 1:10000 to simulate the serological detection of CABYV, MABYV and SABYV in the natural host cucumber.

## Abbreviations

BrYV: Brassica yellow virus; CABYV: Cucurbit aphid-borne yellows virus; CuABYV: Cucumber aphid-borne yellows virus; ELISA: Enzyme-linked Immunosorbent Assay; IPTG: Isopropyl-β-D- thiogalactoside; LABYV: Luffa aphid-borne yellows virus; MABYV: Melon aphid-borne yellows virus; MP: Movement Protein; ORF: Open Reading Frame; PABYV: Pepo aphid-borne yellows virus; PLRV: Potato leafroll virus; RT-PCR: Reverse transcription polymerase chain reaction; SABYV: Suakwa aphid-borne yellows virus; ScYLV: Sugarcane yellow leaf virus; SDS-PAGE: Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis; TBST: Tris-buffered saline; ZABYV; Zucchini aphid-borne yellows virus.

## Declarations

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including 3Flag-MP<sup>BrYV</sup>, 3Flag-MP<sup>PLRV</sup> and 3Flag-MP<sup>ScYLV</sup>. We are grateful to Nitesh Datt for the revision of the manuscript and Zhitong Zhang for the cultivation of *Nicotiana benthamiana* plants.

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## **Competing interests**

The authors declare that they have no competing interests.

## **Availability of data and material**

All data generated or analyzed during this study are included in this published article. Materials are available from the corresponding author on reasonable request.

## **Authors' contributions**

CGH conceived the study and revised the manuscript critically. SKZ, XS and YZL designed and carried out the experiments. SKZ and TYZ drafted the manuscript. YW, ZYZ, DWL and JLY contributed reagents/materials/analysis tools. All authors read and approved the final manuscript.

## **Ethics approval and consent to participate**

Not applicable

## **Consent for publication**

All the participants are consent for publication.

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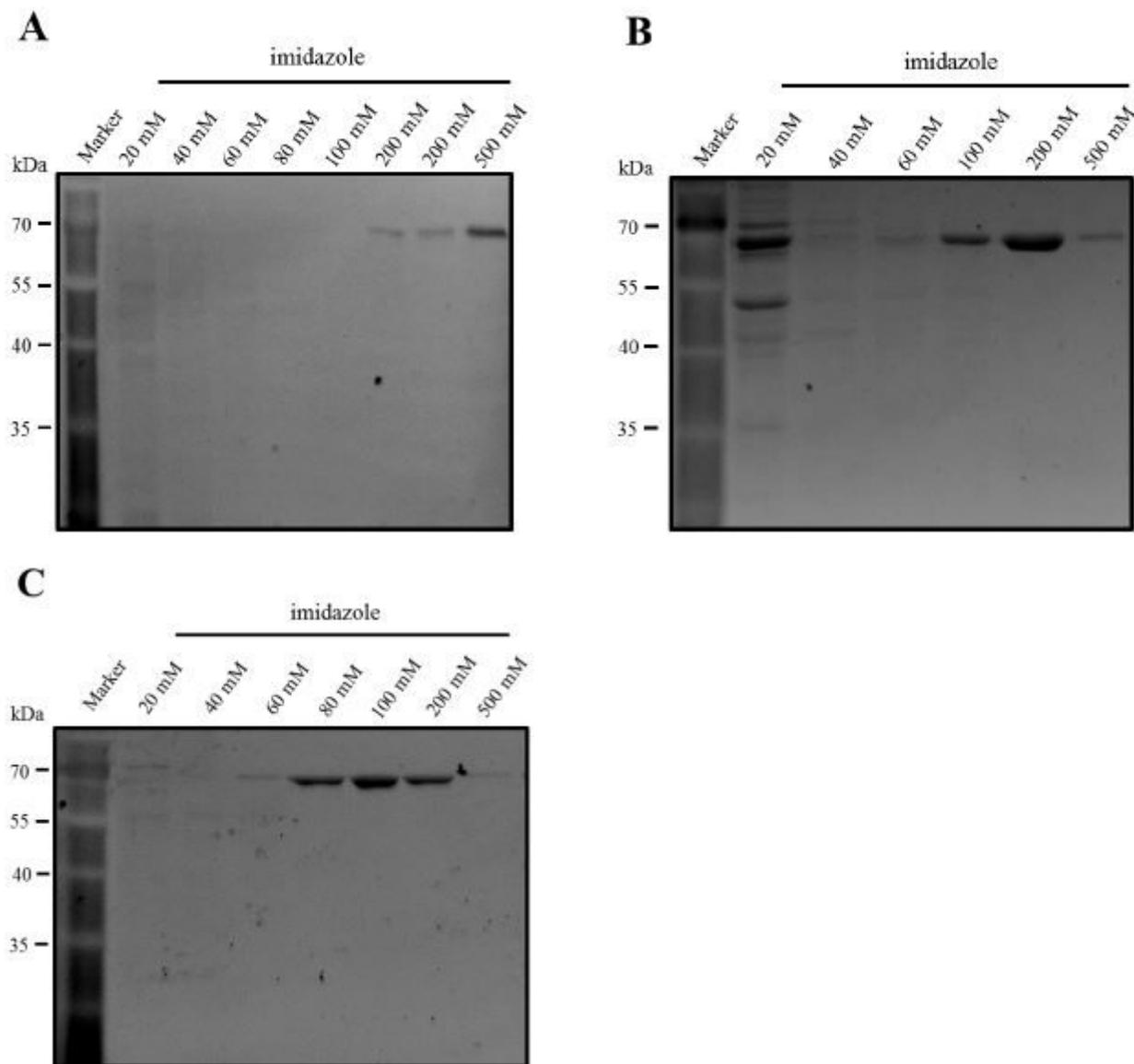
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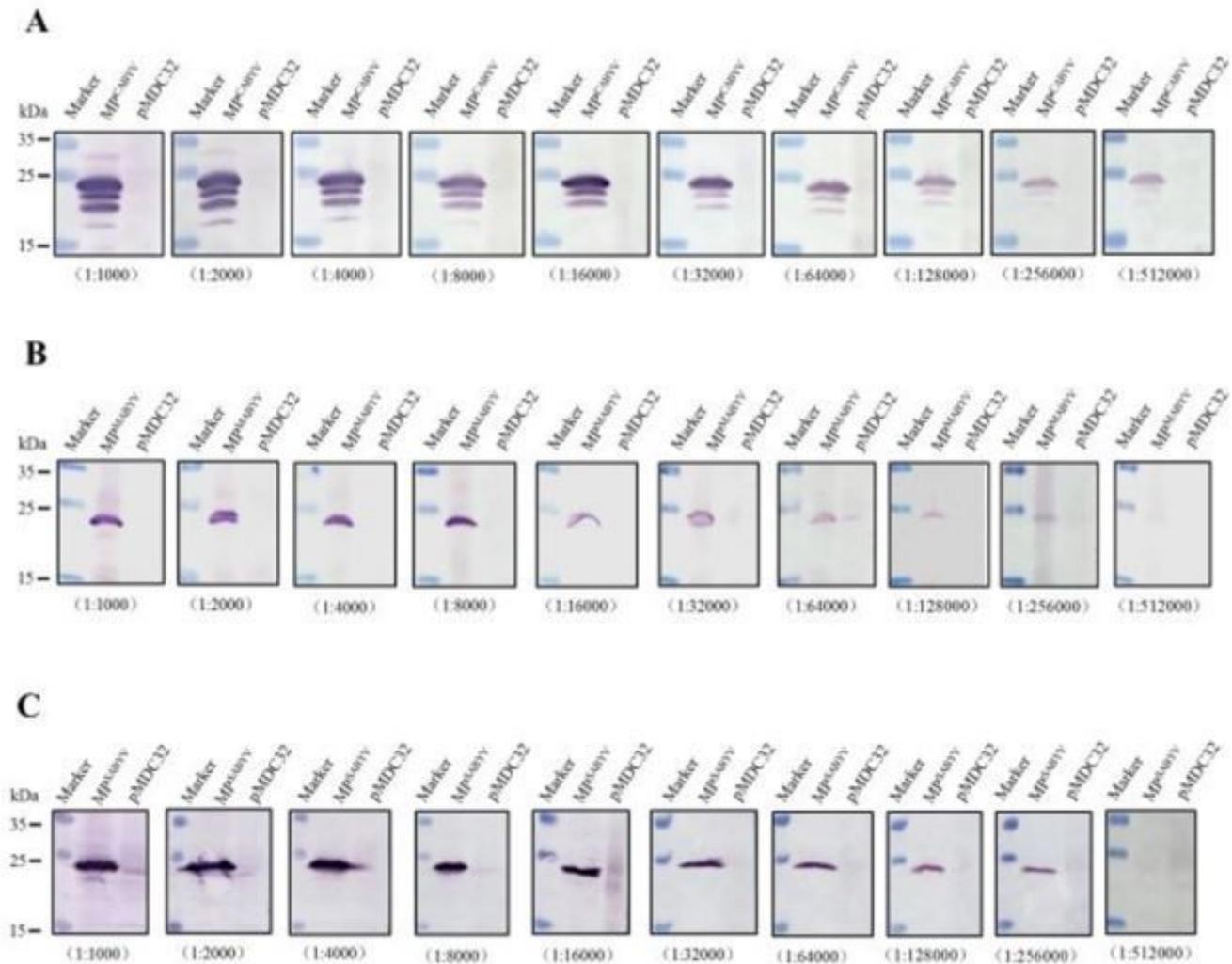
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## Figures



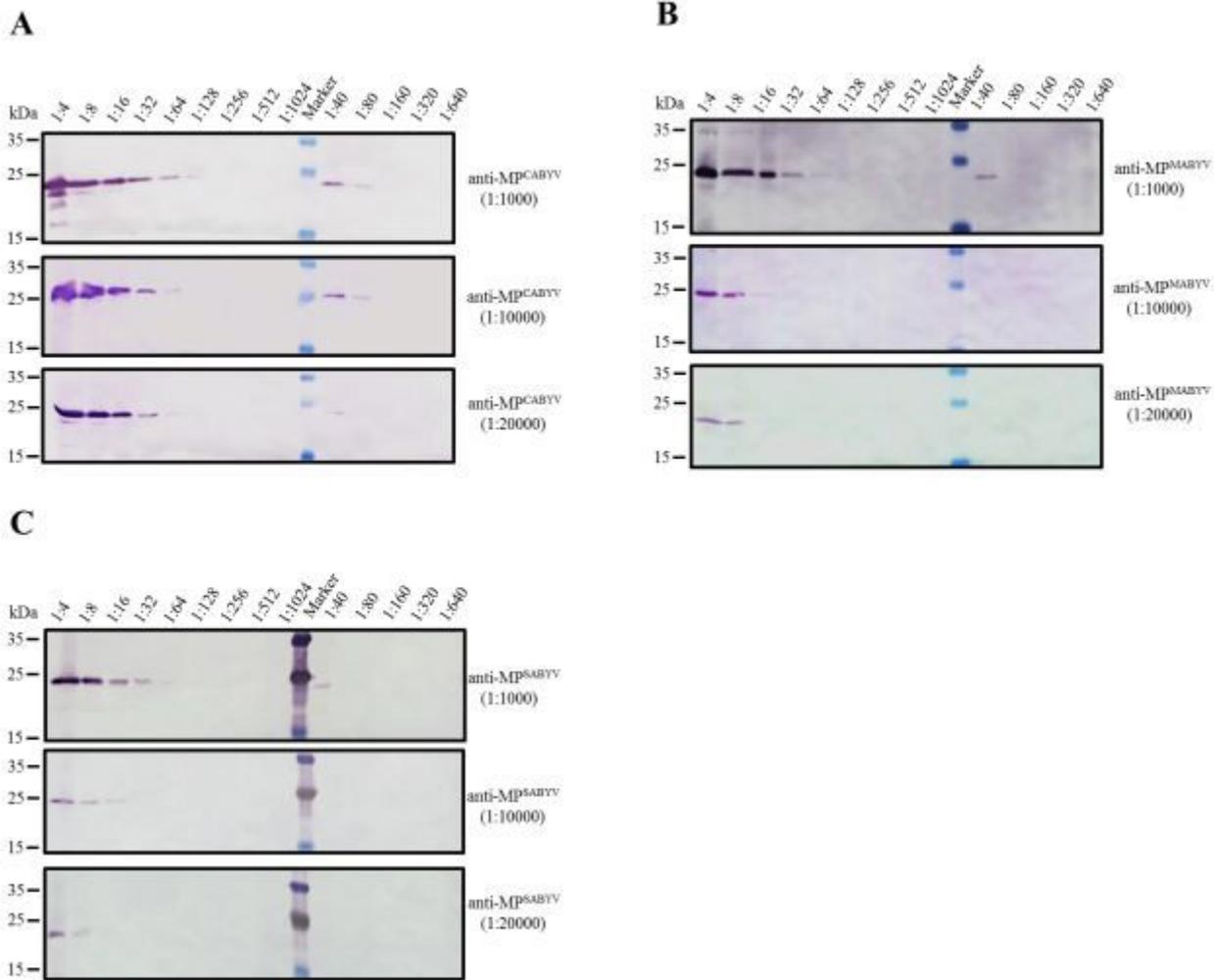
**Figure 1**

SDS-PAGE analysis of CABYV-MP(A), MABYV-MP(B) and SABYV-MP(C) recombinant proteins. The first lane was PageRuler Prestained Protein Ladder (Marker), and the other lanes were fusion proteins eluted with 7 different concentrations of imidazole respectively.



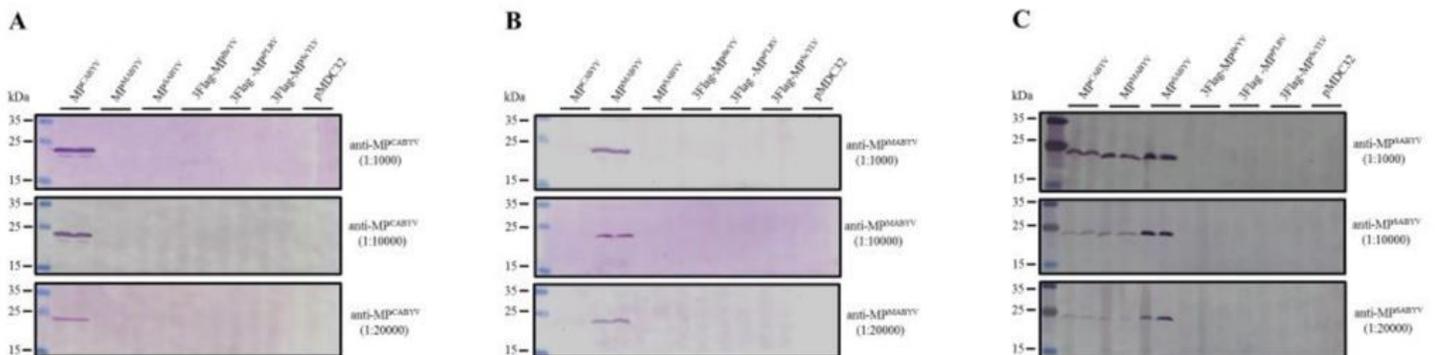
**Figure 2**

Titer determination of the three antisera by Western blotting (A&B&C). All the three antisera were used at 10 different dilutions (1:1000, 1:2000, 1:4000, 1:8000, 1:16000, 1:32000, 1:64000, 1:128000, 1:256000 and 1:512000). Each panel consisted of a left lane of PageRuler Prestained Protein Ladder (Marker), a middle lane containing total proteins extracted from *N. benthamiana* leaves transiently expressed MPCABYV, MPMABYV and MPSABYV protein respectively. pMDC32 empty vector was used as a negative control in the right lane.



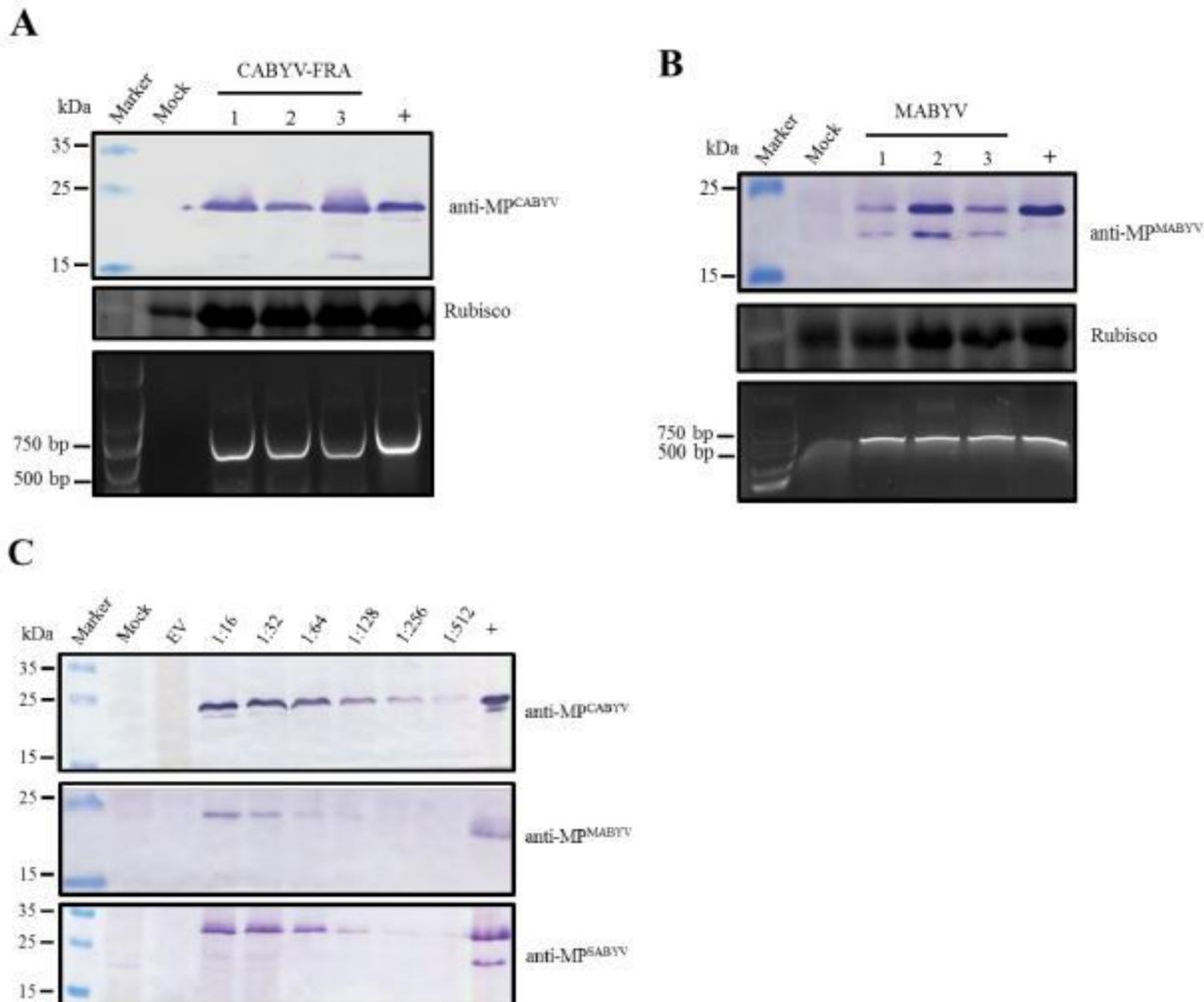
**Figure 3**

Sensitivity analysis of the three antisera by Western blotting. Lane Marker: meaned PageRuler Prestained Protein Ladder, and the rest of the lanes showed two different dilutions of protein samples extracted from *N. benthamiana* leaves (1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:40, 1:80, 1:160, 1:320, 1:640). Anti-MPCABYV (A), anti-MPMABYV (B) and anti-MPSABYV (C) were used at the ratio of 1:1000, 1:10000 and 1:20000, respectively.



**Figure 4**

Specificity analysis of the three antisera by Western blotting. The leftmost lane was PageRuler Prestained Protein Ladder (Marker), and the rest of lanes were proteins extracted from *N. benthamiana* leaves transiently expressing different MPs including CABYV, MABYV, SABYV, BrYV, PLRV and ScYLTV. pMDC32 empty vector was used as a negative control. The three antisera were used at the ratio of 1:1000, 1:10000 and 1:20000 respectively.



**Figure 5**

Applicability analysis of the three antisera by Western blotting. (A and B) Comparison of detections of CABYV and MABYV from *N. benthamiana* leaves inoculated with full-length infectious clone using corresponding antiserum and RT-PCR. Lane Marker: PageRuler Prestained Protein Ladder. Lane Mock: inoculated pCass-RZ empty vector. Lane 1, 2, 3: three separate protein samples expressing CABYV or MABYV infectious clone. Lane "+": protein extracted from *N. benthamiana* leaves transiently expressed MPCABYV and MPMABYV. Rubisco stained with Coomassie brilliant blue was used as a loading control (Middle panel). The lower panel was RT-PCR detection from the same samples. (C) Simulation detection of Cucumber infected with CABYV, MABYV and SABYV. Lane Marker: PageRuler Prestained Protein Ladder. Lane Mock: protein extract from healthy cucumber leaves. Lane EV: *N. benthamiana* leaves inoculated pMDC32 empty vector. Lane "+": protein extracted from *N. benthamiana* leaves transiently

expressing the three MP genes. The rest of the lanes showed multiple dilutions mixed with cucumber protein and *N. benthamiana* protein (1:16, 1:32, 1:64, 1:128, 1:256 and 1:512). The three antisera were used at the ratio of 1:10000 respectively.