Journal of Integrative Agriculture 2017, 16(1): 115–124

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Development and detection application of monoclonal antibodies against *Zucchini yellow mosaic virus*

CHEN Zhe[1], ZHANG Ming-hao[1], ZHOU Xue-ping[1, 2], WU Jian-xiang[1]

State Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, P.R.China

[2] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China

Abstract

Aphid-borne *Zucchini yellow mosaic virus* (ZYMV) is one of the most economically important viruses of cucurbitaceous plants. To survey and control this virus, it is necessary to develop an efficient detection technique. Using purified ZYMV virion and the conventional hybridoma technology, three hybridoma cell lines (16A11, 5A7 and 3B8) secreting monoclonal antibodies (MAbs) against ZYMV Zhejiang isolate were obtained. The working titers of the ascitic fluids secreted by the three hybridoma cell lines were up to 10^{-7} by indirect enzyme-linked immunosorbent assay (ELISA). All MAbs were isotyped as IgG1, kappa light chain. Western blot analysis indicated that the MAb 3B8 could specifically react with the coat protein of ZYMV while MAbs 5A7 and 16A11 reacted strongly with a protein of approximately 51 kDa from the ZYMV-infected leaf tissues. According to this molecular weight, we consider this reactive protein is likely to be the HC-Pro protein. Using these three MAbs, we have now developed five detection assays, i.e., antigen-coated-plate ELISA (ACP-ELISA), dot-ELISA, tissue blot-ELISA, double-antibody sandwich ELISA (DAS-ELISA), and immunocapture-RT-PCR (IC-RT-PCR), for the sensitive, specific, and easy detection of ZYMV. The sensitivity test revealed that ZYMV could be readily detected respectively by ACP-ELISA, dot-ELISA, DAS-ELISA and IC-RT-PCR in 1:163 840, 1:2 560, 1:327 680 and 1:1 310 720 (w/v, g mL^{-1}) diluted crude extracts from the ZYMV-infected plants. We demonstrated in this study that the dot-ELISA could also be used to detect ZYMV in individual viruliferous aphids. A total of 275 cucurbitaceous plant samples collected from the Zhejiang, Jiangsu, Shandong and Hainan provinces, China, were screened for the presence of ZYMV with the described assays. Our results showed that 163 of the 275 samples (59%) were infected with ZYMV. This finding indicates that ZYMV is now widely present in cucurbitaceous crops in China. RT-PCR followed by DNA sequencing and sequence analyses confirmed the accuracy of the five assays. We consider that these detection assays can significantly benefit the control of ZYMV in China.

Keywords: *Zucchini yellow mosaic virus*, monoclonal antibody, ACP-ELISA, dot-ELISA, tissue blot-ELISA, DAS-ELISA, IC-RT-PCR

Received 25 March, 2016 Accepted 24 May, 2016

CHEN Zhe, E-mail: chenzhelily@163.com; Correspondence WU Jian-xiang, Tel: +86-571-88982250, Fax: +86-571-86971498, E-mail: wujx@zju.edu.cn; ZHOU Xue-ping, E-mail: zzhou@zju.edu.cn

doi: 10.1016/S2095-3119(16)61416-8

1. Introduction

Zucchini yellow mosaic virus (ZYMV), a member of the genus *Potyvirus*, is one of the most economically important plant viruses infecting cucurbitaceous crops (Lisa *et al.* 1981; Murphy *et al.* 1995). ZYMV is known to be efficiently

transmitted by aphid in a non-persistent manner. It was also reported to be transmitted worldwide through infected seeds (Lisa *et al.* 1981; Schrijnwerkers *et al.* 1991). Although ZYMV infection was mostly found in the cucurbitaceous crops, it can be readily transmitted to experimental host plants including many species in 11 families of dicotyledons (Lecoq *et al.* 1981; Lisa *et al.* 1981). Disease symptoms caused by ZYMV infection include yellow mottling in leaves, stunting of the plant and fruit distortion. It was reported that ZYMV infection could cause up to 94% loses of crop yield (Desbiez and Lecoq 1997; Simmons *et al.* 2013).

ZYMV virion is flexuous, filamentous and non-enveloped particle with 750 nm in length and 11 nm in diameter. ZYMV genomic RNA is approximately 9 600 nucleotide long and is single-stranded. The 5′ end of the viral RNA is covalently linked with viral protein genome linked (VPg) and the 3′ poly(A) tail is encapsidated by the 36 kDa coat protein (Desbiez and Lecoq 1997). The viral genome contains a single long open reading frame (ORF) that translates a large single polyprotein. This polyprotein is then co- and/or post-translationally cleaved to produce 10 mature proteins (Silvio *et al.* 2001).

So far, several ZYMV detection methods, including a polyclonal antibody based enzyme-linked immunosorbent assay (PAb-ELISA) (Menassa *et al.* 1986; Miroslav *et al.* 2007), dot-ELISA (Somowiyarjo *et al.* 1989), double-antibody sandwich ELISA (DAS-ELISA) (Desbiez *et al.* 1996; Desbiez and Lecoq 1997), transmission electron microscopy (TEM) (Zechmann and Zellnig 2009), reverse transcription-PCR (RT-PCR) (Simmons *et al.* 2013), and RT-loop-mediated isothermal amplification assay (RT-LAMP) (Kuan *et al.* 2014), have been reported. Of these reported assays, the serological assays are the most suitable assays for large-scale detection of the virus during field surveys. In this study, three monoclonal antibodies (MAbs) specific for the ZYMV Zhejiang isolate were produced. Using these three MAbs, five detection methods for ZYMV were developed. Results of field surveys using these newly developed methods demonstrated that ZYMV is now prevalent in cucurbitaceous crops in China.

2. Materials and methods

2.1. Virus sources and field sample collections

ZYMV Zhejiang isolate, *Watermelon mosaic virus* (WMV), *Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus* (CGMMV) and *Potato virus Y* (PVY) were collected from crop fields in China and were characterized by RT-PCR followed by nucleotide sequencing, and then maintained individually in authors' laboratory. Aphids were fed on ZYMV-infected pumpkin plants maintained inside a greenhouse. ZYMV virion was purified from ZYMV-infected pumpkin leaf tissues as described previously (Zhou *et al.* 1994), and then used as an immunogen for MAb preparations. The quality of purified virion was examined under an electron microscope (JEM-1200 EX, JEOL Ltd., Tokyo, Japan) after negative staining with 2% (w/v, g mL^{-1}) phosphotungstic acid (PTA).

A total of 275 cucurbitaceous plant samples were collected from fields in the Zhejiang, Hainan, Jiangsu and Shandong provinces, China, during 2014–2015 and tested for the presence of ZYMV with the methods developed in this study.

2.2. Preparation of MAbs

Animal experiments were carried out using female BALB/c mice provided by the Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (Certificate of animal quality: Zhong Ke Dong Guan No. 003) and at the Research Center of the Laboratory of Animal Science, Zhejiang University of Traditional Chinese Medicine, Hangzhou, China. All experiments were done in accordance with the Principles of the Helsinki accords and approved by the Animal Experimentation Ethics Committee of Zhejiang University, Hangzhou, China.

Purified ZYMV virion was used to immunize three 6-wk-old BALB/c mice as described previously (Wu *et al.* 2014). Production of hybridomas secreting MAbs against ZYMV was performed as described (Shang *et al.* 2011). The hybridomas were then injected into BALB/c mice to produce ascitic fluids and the working titers of the ascitic fluids were individually determined by indirect ELISA. MAbs isotypes were determined using an isotyping kit as instructed (Sigma-Aldrich, St. Louis, MO, USA). The specificity and sensitivity of the MAbs were confirmed by Western blot and ACP-ELISA as described previously (Li *et al.* 2015).

2.3. ACP-ELISA

ACP-ELISA was performed as described (Wu *et al.* 2011) with specific modifications. Crude extracts were prepared from ZYMV-infected (positive) or healthy (negative) cucurbitaceous leaf tissues by grinding the tissues (1 g leaf tissues in 10 mL buffer) in 0.05 mol L^{-1} sodium bicarbonate buffer, pH 9.6. The extracts were centrifuged at 5 000×g for 3 min prior to use. Wells of 96-well microtiter plates were pre-coated with the supernatant from the healthy or ZYMV-infected crude extracts (100 μL per well) for 2 h at 37°C or overnight at 4°C. After three washes with PBST (0.01 mol L^{-1} PBS, 0.05% Tween-20, pH 7.4), the wells were blocked with a blocking buffer (PBS containing 3% dried skimmed milk) (250 μL per well) for 30 min at 37°C. Anti-ZYMV MAbs were