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MONOCLONAL ANTIBODIES TO IDENTIFY TOMATO MOSAIC TOBAMOVIRUS (TOMV)

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ABSTRACT

Monoclonal antibodies were obtained against Tomato mosaic tobamovirus (ToMV) isolated in Brazil. One antibody (8G7G2) isotyped as IgG2b (κ light chain) showed strong specificity and very low cross reaction with the Tobacco mosaic virus (TMV). It can be used in identification of tomato mosaic virus (ToMV).

Key words: Tomato mosaic tobamovirus, ELISA, monoclonal antibodies

INTRODUCTION

The mosaic of tomato crops can be caused by a specific virus species classified in the *Tobamovirus* genus (14). The infected tomato plants show light and dark green mottled areas on the leaves and the fruits may be reduced in size and number with uneven ripening (12). The disease can be disseminated through infected tools and seeds, which control can be more difficult. It is very important in Europe and already do occur in Brazil (1,5). Three Tomato mosaic tobamovirus strains (ToMV) were reported and one have spread out in São Paulo plantations (1), where 30% of the tomato Brazilian production is harvested (6).

The precise identification of ToMV is done by time-consuming differentiation by host plant tests and polyclonal antibody serology, which presents cross-reaction with tobacco mosaic tobamovirus (TMV), which also infects tomato plants. This could be avoided by using highly specific and uniform monoclonal antibodies (MAb) suitable for plant virus diagnosis (5,8,13). This work tested this approach aiming practical uses in selecting monoclonal antibodies to recognize specific ToMV epitopes.

MATERIALS AND METHODS

Samples of ToMV and TMV were CsCl-gradient purified according to Caner *et al.* (2). To produce monoclonal antibodies, six-week-old BALB/c female mice were immunized by one subcutaneous injection of 20 µg of purified ToMV in Freund's Complete Adjuvant, followed by new injections of PBS (8g NaCl; 0.2 g KCl; 1.5 g $Na2HPO_4$ and 0.2 g $KH2PO_4$) every 15 days (7). High antibody titers were obtained after five to six immunizations, and one final injection was done intravenously two days prior to cell fusion. Mice blood samples for titration were taken through the ocular plexus before every injection, centrifuged and the serum stored at -20ºC. Plate trapped antigen enzyme immunoassay/PTA-ELISA was used for antisera titration and positive colony screening (3). Wells of 96-well microtiter plates were coated with 50 µl of antigen solution containing 1 µg/ml purified ToMV after incubation at 37ºC for one hour. Both ToMV and TMV were used in titration of sera culture supernatants. The conjugate was horseradish peroxidase/ortho phenylene diamine dye (OPD) enzyme system in phosphate/citrate buffer. Absorbance measurements were taken after 3-4 minutes at 492 nm (ELISA Reader BioRad, model 550).

The higher antibody titer mouse had the spleen cells fused to the myeloma cell mouse line SP2/Ag14 (7,13). After fusion, cells were distributed over ten 96-well microtiter plates macrophage feeder layered and incubated in 5% CO_2 atmosphere at 37ºC (water jacked chamber, Forma Scientic, model 3158). The feeder layer was harvested from mouse peritoneal macrophage cells in RPMI-1640 medium (Gibco-BRL, 31800-022) and transferred to CO_2 incubator chamber 37ºC for 48 hours. Ten

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days after cell fusion 50 µl of supernatant fluid were taken from the wells for PTA-ELISA assay (3). High titer colonies were expanded over ten 96-well microplates with macrophages feeder layer and indirect ELISA was performed for colony selection. After ten days of growth, ten colonies were cloned and again screened by an indirect ELISA (4). The selected MAbs were isotyped for IgG1, IgG2a, IgG2b, IgM, IgA, *Kappa* and *Lambda* patterns (2,4,7) using a mouse immunoglobulin monoclonal antibody-based isotyping kit system (Gibco-BRL, cat. no. 9660SA). Microplates coated with ToMV were treated with the MAbs ascite obtained (4,15) and purified in sepharose-protein A column of class G specific rabbit polyclonal antibodies (BioRad, cat. no. 153-6153). Purification methods were specific for each immunoglobulin class (14). The procedure is described in the ELISA protocol (3).

RESULTS AND DISCUSSION

According to the methodology for MAbs production against ToMV, six-week-old BALB/c female mice were immunized and the one presenting high antibody OD titer units was sacrificed. Spleen cells from this mouse were fused to the myeloma cell mouse line SP2/Ag14 (7,13). Fused colony cell growth was observed in 452 out of 960 wells of microtiter plates, from which twenty three colonies were selected after ELISA tests (3,4). Four monoclonal antibodies against ToMV with very low cross-reactivity with TMV were selected. TMV is a tobamovirus with very closely related characteristics, including morphology, symptoms in host plants and polyclonal antibodies based ELISA titer (14,16). The titers of 8G7G2, 10H9F9, 8C2F12 and 8C12H12 MAbs are presented in Fig. 1.

The efficiency of MAbs production was quite low and only four interesting MAbs were obtained. Examples like this have been observed elsewhere for quite similar epitopes under scrutiny as observed for detection and characterization of PPV (10) and for WSSMV viruses (9). Where monoclonal antibodies cross-reactivity is detected Western Blot analysis is used as done before to distinguish among prunus virus isolates (PVIS) from plum pox potyvirus (PPV) (10).

The isotype test experiments (2,3,4,7,15) indicated that 8G7G2 MAb belongs to IgG2b *kappa* chain immunoglobulin. The specificity, as estimated from competition assays against ToMV, has shown to be about 20% higher for 80ug and 160ug ascite concentrations (Fig. 2). Therefore some epitopes can also be shared by rabbit anti-serum against ToMV indicating that they are quite reactive. The results also indicate that 10H9F9, 8C2F12 and 8C12H12 antibodies can still be of polyclonal nature although being very specific to ToMV. They should require additional cloning and selection for possible monoclonal production.

Similar isotyping kit results were obtained testing MAbs anti potato S-virus and potato M-virus (11) and wheat yellow mosaic virus (WYMV), where about 80% of the MAbs belong

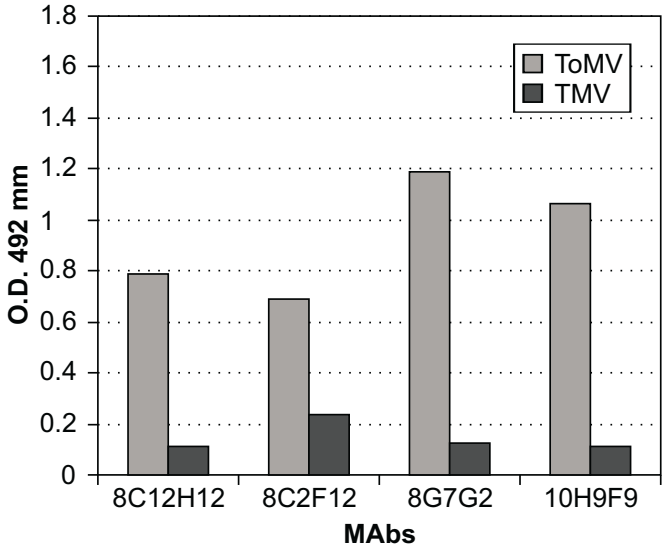

Figure 1. ToMV monoclonal antibodies titers, detected by PTA-ELISA, using microplates coated with 1 µg/ml of ToMV and TMV (O.D. measurements).

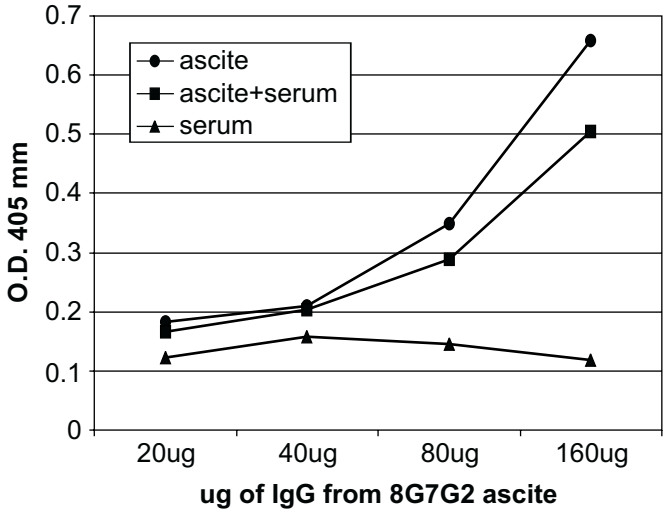

Figure 2. Competition assay using 8G7G2 ascites purified in sepharose-protein A and rabbit anti-serum against ToMV (O.D. measurements).

to IgG sub-classes. They are the best ones as field test for identification of different strains, with the frequency of about 70% of the total IgG cells decreasing cross-reaction with other compounds (9). In conclusion, 8G7G2 monoclonal antibody gives the highest reaction among the four MAbs selected and is undoubtedly able to recognize ToMV with high specificity. This effective MAb against ToMV has an application in field tests aiming future use in virus-free tomato seeds certification.

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RESUMO

Anticorpos monoclonais para identificar o tobamovírus do mosaico do tomateiro (ToMV)

Foram obtidos anticorpos monoclonais contra o vírus do mosaico do tomateiro (ToMV) isolado no Brasil. O anticorpo 8G7G2 isotipado como IgG2b (cadeia leve κ apresentou alta especificidade para o ToMV e baixa reação cruzada com o vírus do mosaico do tabaco (TMV) e poderá ser usado na identificação do ToMV.

Palavras-chave: vírus do mosaico do tomateiro, ELISA, anticorpos monoclonais

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