LETTER TO THE EDITOR

MONOCLONAL ANTIBODY TO OLIVE LATENT VIRUS 1

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Olive is a crop of high economic importance in Portugal. Its susceptibility to virus infections justifies research leading to accurate virus detection. Two necroviruses mechanically transmissible to herbaceous indicator plants have been isolated from symptomatic olive trees: Olive latent virus 1 (OLV-1) and Tobacco necrosis virus D (TNV-D) (*1–5*). OLV-1 was also isolated from citrus trees in Turkey (*6*) and from tulips in Japan (*7*).

In this study, we attempted to prepare a monoclonal antibody (MAb) specific to OLV-1, which could be used in ELISA for accurate diagnostics of this virus. Olive OLV-1 isolates from cv. Galega vulgar (GM6) (*4, 8,* and *9*), cv. Negrinha do Freixo, and cv. Cordovil de Serpa, and tulip OLV-1 isolate Pare-P were used as positive controls in ELISA. Cynosurus mottle virus (CynMV), Coksfoot mild mosaic virus (CMMV), Coksfoot mottle virus (CoMV), Rice yellow mottle virus (RYMV), Southern bean mosaic virus (SBMV) (all members of the genus *Sobemovirus*), and TNV-D (members of the genus *Necrovirus*) isolates GP and DSMZ PV-0192 were used as negative controls. OLV-1 GM6 isolate was maintained in *Nicotiana benthamiana* plants and purified from symptomatic leaves *(5).* The purified virus sedimented as a single band in density gradient, which was infectious for *N. benthamiana* plants. In SDS-PAGE, the purified virus showed a major band of about 30 K corresponding to OLV-1 coat protein (CP) and gave a positive reaction with the polyclonal antiserum to OLV-1 in ELISA. A polyclonal antiserum to OLV-1 GM6 isolate was obtained by immunizing New Zealand rabbits.

To prepare a MAb to OLV-1, a consensus peptide (aa 14–24) was selected from viral CP by multiple alignment (*10*) of sequences of the following necroviruses: OLV-1 (Acc. Nos. NP 043911, CAA59981, and BAB55656); TNV-D (Acc. Nos. BAA00790 and AAC57950), TNV-A (Acc. No. NP 056828), Leek white stripe virus (Acc. No. NP 044744), and Beet black scorch virus (Acc. No. NC 004452). A high hydrophilicity was predicted for the selected peptide FYESGPQRVRT (*11*), whose specificity was confirmed (*12*). Balb/c mice were immunized intraperitoneally with this synthetic peptide coupled with colloidal gold (*13*) and emulsified with MPL®/TDM, poly(A,U) (*14*), and Freund's incomplete adjuvant (all from Sigma). Fusion and selection was made in standard manner (*15*). The presence of MAb in supernatant fluids was detected by ELISA using purified OLV-1 as antigen and alkaline phosphatase-conjugated goat anti-mouse IgM μ-chain or IgG γ-chain (Sigma). From the clones secreting MAbs that reacted positively, the clone 3/19 exhibiting the highest reactivity was selected and recloned twice by limiting dilution method. Western blot analysis

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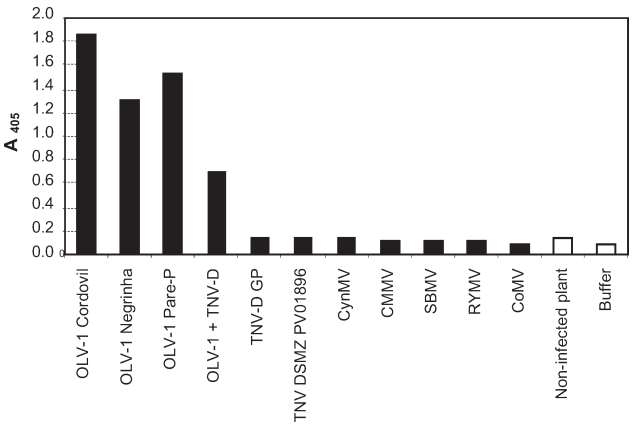
Abbreviations: CMMV = Coksfoot mild mosaic virus; CoMV = Coksfoot mottle virus; CP = coat protein; CynMV = Cynosurus mottle virus; MAb = monoclonal antibody; OLV-1 = Olive latent virus 1; RYMV = Rice yellow mottle virus; SBMV = Southern bean mosaic virus; TAS-ELISA = triple-antibody sandwich ELISA; TNV-A, TNV-D = Tobacco necrosis viruses A, D



confirmed its reactivity with purified OLV-1 (data not shown).

Triple-antibody sandwich ELISA (TAS-ELISA) was used for testing the MAb binding to OLV-1 in plant extracts (*8*). *N. benthamiana* plants infected with various isolates of OLV-1 were used as positive controls, while non-infected *N. benthamiana* plants and other indicator plants infected with other viruses were employed as negative controls (the figure). TAS-ELISA gave A_{405} of 1.3–1.8 for *N. benthamiana* infected with two different olive OLV-1 isolates, and A_{405} of 1.5 for *Tetragonia expansa* infected with tulip OLV-1 isolate. Importantly, the MAb did not cross-react with the extracts of non-infected plants (A_{405} of 0.146) or plants infected with some sobemoviruses or necroviruses (A_{405} of 0.116). A mixed infection with OLV-1 plus TNV-D gave a much lower absorbance compared to single OLV-1 infection, probably due to a lower number of virus (OLV-1) particles in infected cells. TAS-ELISA in comparison to Western blot analysis (data not shown) produced, as a rule, a stronger positive reaction. This discrepancy might suggest that the epitope recognized by the MAb is to certain extent discontinuous and is destroyed in reducing conditions of the SDS-PAGE step of Western blot analysis. Consequently, its ability to give a positive reaction in Western blot analysis is reduced (*16*).

This communication reports on a successful preparation of a MAb specific to OLV-1, achieved for the first time by immunization with a synthetic peptide corresponding to a particular region of viral CP. Due to its strong reactivity in TAS-ELISA it could be used for OLV-1 detection by this method in diseased plant tissues.

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