

SHORT COMMUNICATION

SEQUENCES OF THE N-TERMINI OF COAT PROTEINS OF PORTUGUESE ZUCCHINI YELLOW MOSAIC VIRUS ISOLATES AND OF AN EPITOPE RECOGNIZED BY A MONOCLONAL ANTIBODY**F.H. Cardoso¹, A. Armada¹, A.M. Fonseca², M.T. Santos³, J. Sequeira³,
A. Clemente¹, O. Sequeira³ and C. Novo¹**¹ Instituto Nacional de Engenharia, Tecnologia e Inovação, Departamento de Biotecnologia, Unidade de Tecnologias de Proteínas e Anticorpos Monoclonais, Edifício F, Estrada do Paço do Lumiar 22, 1649-038, Lisboa, Portugal² Universidade do Algarve, Campus de Gambelas, Faro, Portugal³ Estação Agronómica Nacional, Quinta do Marquês 2784-505 Oeiras, Portugal**SUMMARY**

The sequences of the N-terminal 132 nucleotides of the coat protein genes of eleven Portuguese isolates of *Zucchini yellow mosaic virus* (ZYMV) were determined. The nucleotide sequence similarities among the eleven isolates suggested two groups, based on different codon usage for the amino acid residues in seven positions. Comparisons among the encoded amino acid sequences and those previously reported indicated that in this region the Portuguese isolates were identical to isolates from the USA (California), Germany, Italy and France. A monoclonal antibody was produced against a Portuguese isolate that recognized the epitope SGTQPT and cross-reacted with particles of other potyviruses.

Key words: ZYMV, monoclonal antibody, nucleotide sequence, epitope, phage display library.

Zucchini yellow mosaic virus (ZYMV) is a member of the *Potyviridae* family, the largest group of plant-infecting viruses (Shukla and Ward, 1989). ZYMV coat protein (CP) is composed of a 214-amino acid core domain flanked by 43- to 45-amino acid N-terminal domain and a 20-amino acid C-terminal domain (Shukla and Ward, 1989). Different domains have been associated with distinct functions of the CP during the virus life cycle. The conserved core, but not the N- or C-terminus, is required for virus assembly (Jagdish *et al.*, 1991; Dolja *et al.*, 1995; Varrelmann and Maiss, 2000), plasmodesmatal gating (Rojas *et al.*, 1997), and cell-to-cell movement (Dolja *et al.*, 1995). The N-terminus has been shown to assist aphid transmission via its DAG motif (Atreya *et al.*, 1991; Gal-On *et al.*, 1992). Several monoclonal antibodies (Mabs) have been obtained for ZYMV particles that recognise epitopes in the N-terminal region of the CP and in the core region (Kundu *et al.*, 1998; Desbiez *et al.*, 2002).

ZYMV was maintained in *C. sativa* as a propagation host in an insect proof greenhouse and purified from systemically infected leaves according to Fribourg *et al.* (1984). The band containing virus particles was collected and the absence of protein other than CP was checked by SDS-PAGE.

BALB/c mice were immunized with purified ZYMV virus particles and monoclonal antibodies were produced according to Somowiyarjo *et al.* (1988), using Sp2/0 Ag14 myeloma cells. Mabs were isotyped by ELISA using the Mab-based Mouse Ig Isotyping Kit (BD Biosciences Pharmingen, San Diego, CA, USA) and purified by chromatographic methods. The selected Mab 71E2 was used for the detection of virus in infected plant material extracts by ELISA as described by Somowiyarjo *et al.* (1988). Immunocapture RT-PCR was used for CP amplifications using the primers ZY-1 and ZY-2 described by Thomson *et al.* (1995). The RT-PCR products were analysed by electrophoresis in 1% agarose gel and stained with ethidium bromide. The excised DNA band was sequenced on both strands using the Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystem, Foster City, CA, USA). The expected 300 bp band was amplified from samples of all the Portuguese isolates after the IC-RT-PCR reaction. The nucleotide sequences coding for the C-terminus of the polymerase and for the N-terminus of the coat protein (N-CP) were deposited in GenBank (accession numbers AY648578 to AY648588).

Although the deduced amino acid sequences for all the tested Portuguese isolates have the same amino acid sequence in the 44 amino acids of the N-CP, there are nucleotide differences in codons for V7, A8, D9, A12, D26, S30 and V37. Four of the isolates use the codons GTT (V7), GCC (A8), GAC (D9), GCC (A12), GAT (D26) and GTG (V37) in contrast with the other seven isolates which use the codons GTG (V7), GCA (A8), GAT (D9), GCT (A12), GAC (D26) and GTA (V37). The codon usage of GTA for Val seems to be specific for this group and position as no similar codon was found for the other Val residues of the sequenced N-CP from both groups.

Table 1. Multi-alignment between ZYMV coat protein (Portuguese isolate) and isolates from other countries.

Port	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
AAG46239(Spain)	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVVA	AKKDK
CAD31036(Hung)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	PGSGEKTMAA	VTKDK
CAD31057(Hung)	SGTQPTVAD	AGATKKNED	DKGKNRDTG	SGSGEKTMAA	VTKDK
CAD31056(Hung)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAB63753(Hung)	SGTQPTVAD	AGTTKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12314(Slov)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12315(Germ)♣	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
CAD12316(Ital)♣	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
Q89330(Reun I)	SDTQTKEAD	AGAAKRDKDE	EKEKKKDVAS	SSANEKTMTA	TAKDK
P18479(Calif)♣	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
O36979(Sing)	SDTQTREAG	AGASKKDKDE	DKDKKKDVAS	SSASEKAVAT	ATKDK
CAD12313(Aust)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12312(Aust)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12311(Aust)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12310(Aust)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12309(Aust)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
CAD12308(Aust)	SGTQPTVAD	AGATKKNED	DKGKNKDATG	SGSGEKTMAA	VTKDK
AAG46240(France)♣	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
AAG46241(France)♣	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
AAG46230(France)	SGTQPAVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVVA	AKKDK
AAG46231(France)	SGTQPAVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVVA	AKKDK
AAG46232(France)	SGTQPAVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVVA	AKKDK
AAG46233(France)♣	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKTVAA	VTKDK
AAG46237(France)	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKIVAA	ATKDK
AAG46238(France)	SGTQPTVAD	AGATKKDKED	DKGKNKDVTG	SGSGEKIVAA	ATKDK
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Conserved residues in all sequences are in bold and marked (*). Sequences 100% homologous to Portuguese isolate are marked (♣). Accession codes are indicated.

Comparison of this sequence with those of other already described ZYMV using ClustalW (Thompson *et al.*, 1994) (Table 1) revealed a 100% amino acid identity with Californian (USA) (P18479), German (CAD12315), Italian (CAD12316) and French isolates (AAG46240, 46241 and AAG46233). The most dissimilar were the sequences of the isolates from Singapore (O36979) (60% identity) and Reunion Island (Q89330) (62% identity). All Portuguese ZYMV isolates gave a positive reaction in ELISA using the purified Mab 71E2. Cross reaction studies with other potyviruses showed that Mab71E2 also recognises *Papaya ring spot virus* (PRSV) (AAR23797), *Potato virus A* (PVA) (NP_734368), *Soybean mosaic virus* (SMV) (CAE76638), *Watermelon mosaic virus* (WMV) (AAR27346), *Bean common mosaic virus* (BCMV) (NP_734358), *Tobacco etch virus* (NP_734206) and *Onion yellow dwarf virus* (AAQ 91384), *Lettuce mosaic virus* (NP_734162).

Peptides that can mimic structural features recognized by Mab71E2 were screened on a 12-mer phage displayed random peptide library (BioLabs, Beverly, Madison, USA) in accordance with manufacturer's instructions. A total of 11 clones from isolated plaques from the third round of panning that were positive in the immunoblot were amplified by PCR and their DNA were sequenced. Selected se-

quences aligned with the sequence of the Portuguese ZYMV isolate indicate that the epitope for this antibody spans the N-CP of ZYMV and its amino acid sequence is SGTQPT, the same epitope recognized by Mab CC11, AE11 (Desbiez *et al.*, 2002). The bulk of the binding energy was supplied by the 6 residues (1-[S,A]GTXPT-6). As the SGTQPT motif is common to all Hungarian (CAD31036, 31057, 31056 and CAB63753), Slovenian (CAD12314), German, Italian, Austrian (CAD12308 to 12313), Spanish (AAG46239) and French (AAG46240, 46241, AAG46233, 46237 and 46238) isolates, recognition of these isolates by Mab 71E2 was predicted. However, Mab 71E2 also recognises PRSV (AAR23797), PVA (NP_734368), SMV (CAE76638) and WMV (AAR27346). Comparisons among peptides from the equivalent region of other potyvirus isolates with the SGTQPT peptide (Table 2) showed a conserved peptide pattern sequence NGTSP[N,D], except for *Bean Yellow Mosaic virus* (BYMV) (NP_734182) with the change Pro to Gly and *Maize dwarf mosaic virus* (NP_734152) with the change Thr to Cys. This amino acid sequence partially overlays one of the preferential immunogenic regions described by Fernandez-Fernandez *et al.* (2002) (TSPNINGM) located in the core region of the coat protein of *Plum pox virus*.

Table 2. Reaction of monoclonal antibody 71E2 with extracts from plants infected with different potyviruses in Indirect ELISA and amino acid sequence deduced for the epitope of the virus coat protein recognized by Mab 71E2.

Antigen / Accession code	Epitope sequence	ELISA reaction (Abs _{405 nm})
<i>Zucchini yellow mosaic virus</i> (Portugal)	SGTQPT	+++
<i>Bean Yellow Mosaic virus</i> (NP_734182)	NGTSGD	-
<i>Lettuce mosaic virus</i> (NP_734162)	NGTSPN	++
<i>Leek yellow stripe potyvirus</i> (NP_734102)	NGTSPN	-
<i>Maize dwarf mosaic virus</i> (NP_734152)	NGCSPN	-
<i>Onion yellow dwarf virus</i> (AAQ91384)	NGTSPN	+
<i>Papaya ring spot virus</i> (AAR23797)	NGTSPD	+
<i>Pea seed-borne mosaic virus</i> (NP_734428)	NGTSPN	-
<i>Potato virus A</i> (NP_734368)	NGTSPD	+
<i>Potato virus Y</i> (CAE46156)	NGTSPN	-
<i>Soybean mosaic virus</i> (CAE76638)	NGTSPD	+
<i>Tobacco etch virus</i> (NP_734206)	NGTSPN	+
<i>Watermelon mosaic virus</i> (AAR27346)	NGTSPD	+
<i>Peper veinal mottle virus</i> (AAB39852)	NGTSPN	-
<i>Bean common mosaic virus</i> (NP_734358)	NGTSPD * * *	+
<i>Zucchini yellow mosaic virus</i> (Bioreba)	nd	+++
<i>Cucumis sativus</i>	na	-
<i>Nicotiana tabacum</i>	na	-
<i>Brasica chinesis</i>	na	-
<i>Chenopodium quinoa</i>	na	-

Alignment made using ClustalW. Accession codes are indicated. Plant extracts (diluted 1/10) of infected versus healthy plants: *Cucumis sativus*, *Nicotiana tabacum*, *Brasica chinesis*, *Chenopodium quinoa*. Samples are considered to be positive when the OD value is equal or higher than three times that of the healthy control. (nd) not determined, (na) not applicable. * Conserved residues, * Almost identical to the ZYMV (Port) epitope sequence. Abs at $\lambda=405$ nm <0.1 (-), 0.12< (+) <0.30, 0.30< (++) <0.7 and >0.7 (+++). ZYMV positive control was supplied by Bioreba (Reinach, Switzerland) and the other potyviruses were purchased from BioRad Laboratories (Hercules, CA, USA).

Mab 71E2 gave a ELISA negative reaction with BYMV (NP_734182). The equivalent epitope in this case is NGTSGD. The presence of Asp as the 6th residue of the epitope suggested that Mab 71E2 would bind, as for most of other potyviruses tested with Asp in that position. The change of a Pro residue for Gly in the 5th position of the epitope could explain the lack of reaction. These results suggest that the amino acid sequence pattern of the epitope recognized by Mab 71E2 is [S,A,N]-G-T-[Q,S]-P-[T,D]. All 5 tested viruses possessing the epitope pattern NGTSPD gave a positive reaction with Mab 71E2. However, of 8 tested viruses possessing the epitope pattern NGTSPN, 5 gave a negative reaction with Mab 71E2, as predicted, but the other 3 gave positive reactions. One possible explanation is the different spatial organization of the epitope NGTSPD. Search for pattern [S,A,N]-G-T-[Q,S]-P-[T,D] in a non-redundant protein database using the program PATTINPROT (Combet *et al.*, 2000) detected among plant virus sequences two of the possible pattern combinations: SGTQPT from the N-CP of ZYMV isolates and NGTSPD located in the core region of the coat protein, for the other tested viruses, PRSV, PVA, WMV and BCMV, as expected.

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