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Coat protein gene sequences of garlic and onion isolates of the onion yellow dwarf potyvirus (OYDV)

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Summary. Partial genomic sequences from an unknown garlic potyvirus and from an onion isolate of the onion yellow dwarf potyvirus (OYDV) were obtained. Comparison of the deduced amino acid sequences showed a similarity of 88% between the respective viral coat proteins. The garlic potyvirus coat protein was expressed in *E. coli* cells, purified, and subjected to Western blot analysis using antibodies raised against different garlic-infecting viruses. The expression protein was consistently recognised by anti-OYDV antibodies and did not react with antibodies specific for leek yellow stripe potyvirus (LYSV), garlic common latent carlavirus (GCLV) and shallot latent carlavirus (SLV). Besides, the garlic potyvirus coat protein was obtained as a fusion protein and used as antigen to produce polyclonal antibodies. These antibodies reacted with purified OYDV virions, but failed to recognise LYSV particles. In the light of this evidence the garlic potyvirus was identified as the garlic strain of OYDV.

Introduction

Commercial cultivars of *Allium sativum* are strictly apomixic and are vegetatively propagated by means of bulbs. This practice almost inevitably leads to the accumulation of viruses in reproductive materials, facilitating their dissemination and producing considerable yield losses [36]. Disease is usually induced by the simultaneous infection of several partially characterised viruses belonging to different taxonomic groups which are collectively known as the garlic viral complex [6, 11, 15, 21, 35]. Typical symptoms include mosaics of different intensities, yellowing, and marked reductions in bulb weight and size [36]. The contribution of each individual component to disease expression remains largely unclear.

Components of the garlic viral complex have been difficult to isolate because they do not infect (or infect very poorly) other plant species. This poses a serious limitation to obtaining homogenous viral preparations and, in turn, complicates the development of antibodies specific for each particular virus. By contrast, the use of antibodies reacting against more than one viral species has contributed often to confusion of taxonomic description. Thus, both the lack of alternative host plants and the scarcity of reliable antisera precluded description of this viral complex using the classical approaches of plant virology.

The best characterised garlic-infecting viruses are onion yellow dwarf potyvirus (OYDV), leek yellow stripe potyvirus (LYSV) and garlic common latent carlavirus (GCLV) [5, 17, 19, 33]. Garlic-specific strains – differing in their host specificities from their onion and leek counterparts – have been described for OYDV and LYSV, and were respectively named OYDV-G and LYSV-G [33]. In addition to these, a variable number of poty- and carlaviruses have been reported [1, 2, 20, 24, 26, 30], but their identity remains obscure and they probably represent mixtures of well known viruses or their strains [30, 31, 32, 33, 34]. More recently, partial genomic sequences from several rod-shaped flexuous viruses [30, 31], and from two potyviruses and one carlavirus [26] have been obtained. One of these potyviruses was identified as LYSV [31, 37]. The composition of the garlic viral complex varies with geographical localisation [35]. Analyses performed with field samples collected in different garlic-producing areas showed that the incidence of OYDV ranges from 52 to 86% [33]. In Argentina, garlic infections by several poty-, carla- and mite-borne-viruses have been reported since 1990 [12, 14]. In these studies, infections by OYDV were found to affect 56–92% of plants sampled in the field [14].

Due to the problems mentioned above, the identity of individual garlic viruses remains largely unclear. In this paper, we report the cloning of a garlic potyvirus that, on the basis of coat protein [28] and 3′ non-coding [16] sequence comparisons and immunological evidence, was identified as the garlic strain of OYDV.

Materials and methods

Plant material and virus strains

Healthy garlic plants were obtained from in vitro-cultured meristem tips that had been previously subjected to thermotherapy [13]. Virus infection was conducted using a single aphid (*Myzus persicae*) that was previously fed on infected garlic plants for 60 sec. After development of symptoms, plants were tested with antibodies reacting against OYDV, LYSV and carnation latent carlavirus (CLV) in ELISA and IEM-D assays. Plants reacting exclusively with anti-OYDV antiserum were selected for further experiments. *A. cepa* cv. Ebenezer plants were mechanically inoculated with OYDV-infected onion leaf extracts in 50 mM $Na_2B_4O_7$, pH 8. LYSV was obtained from leek plants (*A. ampeloprasum var. porrum* cv. Carentam) sampled from the field. Plants showing typical LYSV symptoms were tested with antibodies against LYSV, OYDV and shallot latent carlavirus (SLV) in IEM-D assays. Only LYSV-positive plants were considered. The OYDV onion isolate used in this paper is an uncoded French isolate obtained by Dr. H. Lot.

Virus purification

Large-scale virion purifications were performed according to the procedure reported by Murphy [25], with minor modifications. Genomic RNA was extracted from viral particles as described by Hull [18]. Virus purification for Western blot assays was performed as follows: 10 to 20 g of onion or leek leaves were homogenised in 2 vol of 0.5 M $Na_2B_4O_7$, 0.2% thioglycolic acid, pH 8. Then, half a volume of $CHCl_3$ was added and thoroughly mixed. After centrifugation in a GSA rotor (Sorvall, USA) for 15 min at 7 000 rpm, the aqueous phase was loaded in a 20% sucrose cushion and centrifuged in a TST-28.38 rotor (Kontron, Italy) for 1 h at 27 000 rpm. The pellets containing purified virions were resuspended in 100 µl of 0.05 M $Na_2B_4O_7$, pH 8, and mixed with the same volume of loading buffer (60 mM Tris-HCl, pH 6.8, 10% glycerol, 5% β-mercaptoethanol, 0.1% SDS). Samples were analysed by SDS-PAGE in a 12% gel.

Molecular cloning

Ten plants of *Allium sativum* (cv. Rosado Paraguayo) showing profuse viral symptomatology were taken from a garlic-producing field in Córdoba (central Argentina). Leaves showing mosaic and stripes were used as a source to obtain a mix of flexuous and rod-shaped viral particles. Genomic RNA extracted from this viral preparation was used as template for cDNA production. Oligo(dT)-primed cDNA synthesis was conducted using a commercial kit (Librarian, Invitrogene, USA). After completion of the reaction, the viral cDNA was ligated to *Bst* XI adaptors and cloned into the pcDNA II vector (Invitrogen, USA). Recombinant plasmids were introduced into JM109 *E. coli* cells and a cDNA library of about one thousand clones was generated. A sequence corresponding to the 3′ genomic region of a potyvirus (tentatively named GP1) was obtained from some of these clones. Two oligonucleotide primers (5′-GGAATTCACAATGGGAGATGGGGAG-3′ and 5′-GTTGTAG-CCAGATCAAGCTTCGTACC-3′) were designed in order to amplify the GP1 coat protein gene and generate *Eco* RI and *Hind* III sites at its 5′ and 3′ ends, respectively. PCR amplification was conducted in a 100 µl reaction mix containing 1 ng of cDNA, 1.5 mM of $MgCl_2$ and 200 ng of each primer. Nucleotide buffer and *Taq* polymerase were purchased from Promega (USA). After 5 cycles of 1 min at 94 °C, 1 min at 38 °C, 2 min at 72 °C, and 30 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C in a DNA Thermal Cycler 480 (Perkin Elmer, USA), a 10 µl aliquot was electrophoresed in a 1.2% agarose gel. The reaction products were visualised by staining with ethidium bromide and the PCR fragment was purified using Wizard PCR Preps (Promega, USA). Then, the fragment was digested with *Eco* RI and *Hind* III and subcloned by cohesive ligation into the expression vectors p-MALc [22] and pKK223-3 [9], which respectively allow the production of GP1 CP as a C-terminal fusion to maltose binding protein (MBP) or as an unmodified protein. The CP gene and 3′ non-translated region of OYDV (onion isolate) were amplified by reverse-PCR using purified genomic RNA as template. In order to introduce a *Not* I site at 3′ end of the PCR fragment, reaction was primed with the oligonucleotides 5′-GATCCTTA-TGAGGTGCACCATCAAGCA-3′ and $(dT)_{18}$ *Not* I (Pharmacia, Sweden). For amplification, a program using 5 cycles of 1 min at 94 °C, 1 min at 45 °C, 2 min at 72 °C, 5 cycles of 1 min at 94 °C, 1 min at 38 °C, 2 min at 72 °C and 30 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C was carried out. The amplification product was electrophoresed in a 1.2% agarose gel and purified as described before. After restriction with *Not* I, the OYDV sequence was ligated to the *Eco* RV and *Not* I sites of the commercial vector $pBSSK^+$ (Stratagene, USA).

Viral protein expression and purification from bacterial cells

JM109 *E. coli* cells were transformed with p-MALc and pKK233-3 vectors carrying the GP1 CP gene. In both systems, expression of the recombinant protein was induced by addition of 0.3 mM IPTG to the culture media. After incubation for 2 h, expression products were analysed by Western blot assays using the appropriate antibodies. The fusion protein expressed from p-MALc (MBP/GP1) was purified from IPTG-induced cell extracts as follows. Bacterial cells were resuspended in lysis buffer (10 mM Na_2HPO_4, 30 mM NaCl, 0.25% Tween 20, 10 mM β-mercaptoethanol and 10 mM EDTA) and disrupted by sonication for 2 min. After centrifugation at 9 000 **g** for 10 min, the supernatants were loaded into an amylose-conjugated affinity chromatography column (1.5 ml bed column). The column was then washed with 10 volumes of buffer (20 mM Tris-HCl, pH 7.4, 0.2 M NaCl) to eliminate non-specific ligands, and eluted with the same buffer supplemented with 10 mM β-mercapto-ethanol and 10 mM maltose to extract the MBP/GP1 protein.

DNA sequencing

Nucleotide sequencing was performed by the dideoxy chain-termination method [27] using a commercial kit (Sequenase, USB, USA). The reaction mixes included either single or double strand DNA, and the sequencing procedure was carried out according to the instructions provided by the manufacturer. The sequences reported in this paper are enlisted under the GeneBank accession No. X89402 (garlic isolate) and X95874 (onion isolate).

Recombinant DNA techniques

DNA digestions, electrophoresis, hybridizations and other DNA manipulations were performed as described in Maniatis et al. [23] and Ausubel et al. [4].

Antisera

Anti-OYDV antibodies were kindly provided by Dr. K. Graichen (Institute fur Pathogendiagnostik, Germany), Dr. D. Maat (Research Institute for Plant Protection, The Netherlands), and Dr. L. Mayoral (IVIC, Venezuela). Anti-LYSV antibodies were prepared by standard procedures in IFFIVE-INTA and INRA-Montfavet, France. Antisera against SLV, garlic common latent carlavirus (GCLV) and carnation latent carlavirus (CLV) were obtained from Dr. D. Maat, Dr. K. Graichen and Dr. E. Luisoni (Instituto di Fitovirologia Applicata, Italy), respectively. Suppliers of antisera used in each assay are mentioned in the legends of the respective figures.

Rabbit immunisation

1 mg of purified MBP/GP1 protein was emulsified with 1 ml of complete Freund's adjuvant. Rabbits (New Zealand) were intradermally injected 10 to 15 times with 100 μl each. The animals were bled 4 weeks later.

Western blots

Leaf tissue was extracted in 60 mM Tris-HCl, pH 6.8, 10% glycerol, 5% β-mercaptoethanol, 0.1% SDS. Samples were boiled for 3 min and then centrifuged for 2 min at 13 000 rpm in a microcentrifuge. Protein content of the supernatant was determined according to Bradford [8]. Samples containing 30–40 μg of soluble protein were electrophoresed in 12% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Viral proteins were detected

by a first incubation with the appropriate antibodies, followed by several washes with 50 mM Tris-HCl, pH 8, 150 mM NaCl, 0.05% Tween 20, and a second incubation step with alkaline phosphatase-linked goat anti-rabbit antibody (Jackson Ltd., UK). After a final wash, phosphatase activity was revealed by a chromogenic reaction using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Sigma, USA) as substrates.

Results

Viral RNA extracted from a mix of purified virions was used as template to obtain a cDNA library representing several garlic viruses. Partial sequencing of size-selected clones showed that three of them (pCC5, pCC6 and pCC15) contained fragments that had 91% sequence homology with garlic virus A (GarV), a novel rod-shaped virus isolated by Sumi et al. [30]. Four other clones (pCP1, pCP6, pCP33 and pCP44) contained sequence motifs that are highly conserved in potyviral coat protein (CP) genes. Three of these clones, pCP6, pCP33 and pCP44, derived from the same genomic sequence. Clones pCP6 and pCP44, respectively encompassing about 1 600 and 3 500 bp, contained partially overlapping cDNA fragments. Sequencing of these fragments led to the assembly of a 1 180 nt potyviral sequence encoding part of the NIa gene, the complete CP gene and the 3′ non-translated region (Fig. 1). Since this sequence was not previously included in public databases, the corresponding virus was provisionally named GP1, for "garlic potyvirus 1". The translated GP1 amino acid sequence showed a similarity of 57.4% in comparison with the corresponding sequence of potato virus Y (PVY [7]). In particular, the GP1 CP N-terminus is 11 amino acids shorter than that of PVY and contains the sequence motif DAG, which is associated to aphid transmissibility [3].

To further explore GP1's identity, the sequence corresponding to the entire CP was amplified by PCR and subcloned into the bacterial expression vectors pKK223-3 and pMALc (plasmids pKK/GP1 and pMAL/GP1). Introduction of pKK/GP1 and pMAL/GP1 in *E. coli* cells respectively led to the production of a 20 kDa polypeptide and a 70 kDa fusion protein (MBP/GP1). Extracts obtained from both expression systems were probed in Western blot assays with antibodies raised against several viruses that reportedly or presumably infect garlic [14]. Both the 20 kDa polypeptide and the 70 kDa fusion protein were recognised by anti-OYDV antibodies. A faint band of about 30 kDa (the MW expected for the GP1 CP sequence) was also recognised in some pKK/GP1 extracts, suggesting that the 20 kDa polypeptide results from intracellular proteolytic processing (Fig. 2). In contrast, these polypeptides did not react with antibodies directed against LYSV, SLV or GCLV. To confirm these results, the MBP/GP1 fusion protein was purified and injected into rabbits to raise polyclonal antibodies. Since OYDV and LYSV are the potyviruses most frequently infecting garlic in Argentina, anti-MBP/GP1 antibodies were used to probe purified OYDV-O, OYDV-G and LYSV virions in Western blot assays. A band of about 30 kDa was specifically recognised by both the anti-OYDV-O and anti-MBP/GP1 antibodies in samples of OYDV-O and OYDV-G virions. In contrast, the anti-MBP/GP1 antibody did not react with a LYSV viral

```
5'ATGGTTGTTGGAGCAAGCCCCATACAATGAGATAGCTCGAAATGGGAAGGCTCCTTATATAGCAGAGACA
   W  L  L  E  Q  A  P  Y  N  E  I  A  R  N  G  K  A  P  Y  I  A  E  T

GCTCTTAAGAAATTATTTACAGGAACCGACGCAAGTGAAGCCGAGTTGGAACGATATTACACGATGTATTTG
 A  L  K  K  L  F  T  G  T  D  A  S  E  A  E  L  E  R  Y  Y  T  M  Y  L

GAACTTGAAAATCAGGAAACGGCTATAAAAGAGGTTCGCTATCAAGCAGGAGATGGGGAGGACGCAGCAGCA
 E  L  E  N  Q  E  T  A  I  K  E  V  R  Y  Q/ A  G  D  G  E  D  A  A  A

CAATCAAATACATCAAAACAAGTTTCGAAGCAGAAGGATAAAGATGTCGATGCAGGCACAACCGGAAAATTC
 Q  S  N  T  S  K  Q  V  S  K  Q  K  D  K  D  V  D  A  G  T  T  G  K  F

ACAGTGCCAAGAATTAAAGCATTGTCTGACAAAATGCGCTTTCCGAAAGTTGGTAAAAGCGTAGTTCTCAAT
 T  V  P  R  I  K  A  L  S  D  K  M  R  F  P  K  V  G  K  S  V  V  L  N

GCGGACGACTTGTTGGCATACAAGCCAGATCAAATTGAATTATACAACACACGAGCAACACAACAACAATTT
 A  D  D  L  L  A  Y  K  P  D  Q  I  E  L  Y  N  T  R  A  T  Q  Q  Q  F

GAAAATTGGTTTGGTGCGATCAAAAAGGAATATGACGTGAATGACGAACAAATGAAAATAATACTGAACGGA
 E  N  W  F  G  A  I  K  K  E  Y  D  V  N  D  E  Q  M  K  I  I  L  N  G

TTGATGGTTTGGTGTATTGAGAACGGCACGTCCCCAAATTTATCAGGTAATTGGACTATGATGGACGGTGAC
 L  M  V  W  C  I  E  N  G  T  S  P  N  L  S  G  N  W  T  M  M  D  G  D

GAGCAGGTTGAATACCCCTTGGCACCGATTCTGGACAACGCAAAACCAACGTTCAGACAAATAATGGCACAT
 E  Q  V  E  Y  P  L  A  P  I  L  D  N  A  K  P  T  F  R  Q  I  M  A  H

TTCAGTGACGCAGCTGAAGCGTACATTGAGTATAGAAATGCCACTGAAAAATACATGCCCCGGTATGGACTT
 F  S  D  A  A  E  A  Y  I  E  Y  R  N  A  T  E  K  Y  M  P  R  Y  G  L

CAGCGAAATCTAACAGAACTAAGTTTAGCACGTTACGCATTCGATTTTTACGAAATGACTTCAAAGACTCCC
 Q  R  N  L  T  E  L  S  L  A  R  Y  A  F  D  F  Y  E  M  T  S  K  T  P

AAACGAGCTAAAGAAGCACATATGCAAATGAAGGCGGCGGCGGTTAGAGGGGCAACTAATCGTTTGTTTGGC
 K  R  A  K  E  A  H  M  Q  M  K  A  A  A  V  R  G  A  T  N  R  L  F  G

CTGGATGGTAACGTAAACACCACGGAAGAGGACACGGAAAGACACACAGCAGCGGACGTGAACAAAAACCAG
 L  D  G  N  V  N  T  T  E  E  D  T  E  R  H  T  A  A  D  V  N  K  N  Q

CACACGTTGCTTGGTATTAGAATGTAACGCCAGTTGTATGTCTTTAGTTTTATATGTACCTTATATAAAAA
 H  T  L  L  G  I  R  M  *

CGCACTTAGTATGTATTCCTCTCTACTTCGTTTAACCTGACTTCGAGCAGCGAAATGTGGTGTACCACTAGT
TGTGGCTGAACGATACGGTTAAATATCTGTGATTAATGTCGGGCTTCATGAGAGTGACGTATCACGAGGTGA
CTCTTGGCGTGAATTACAGAGACAAAAAAAA 3'
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Fig. 1. Nucleotide and translated amino acid sequences deduced from the GP1 cDNA clone. The proteolytic cleavage site between the NIa and CP sequences, and the aphid transmissibility motif are underlined in the amino acid sequence. The polyprotein stop codon is denoted by an asterisk

preparation (Fig. 3A). Noteworthy, in these Western blot assays the OYDV-G CP displayed a lower electrophoretic mobility than the OYDV-O CP. Similar results were obtained when these analyses were performed on leaf extracts of healthy, OYDV- and LYSV-infected garlic and leek plants (Fig. 3B). In this case, a second band of lower MW was evidenced in OYDV-infected extracts, presumably arising from partial proteolysis of the OYDV CP during the extraction procedure.

These results prompted us to clone and sequence the corresponding genomic region of a well characterised onion isolate of OYDV (OYDV-O; [15]). A reverse-PCR was performed using as template RNA from purified OYDV-O virions and two oligonucleotide primers containing a poly(A) tract and the 27 nt sequence immediately upstream of the PVY CP gene, respectively. A PCR

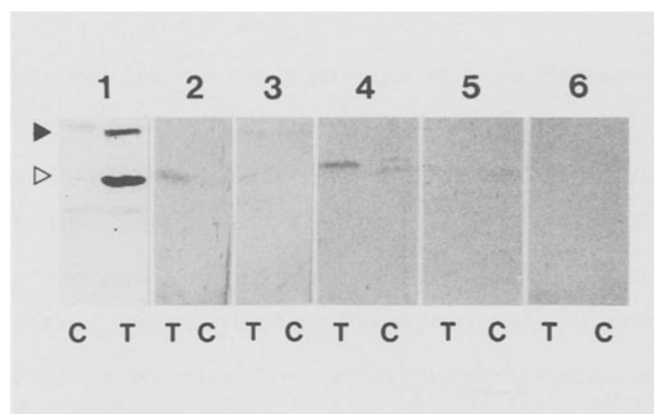

Fig. 2. Western blot analysis of the GP1 polypeptide. Extracts from IPTG-induced *E. coli* cells transformed with pKK/GP1 (*T*) and pKK223-3 (*C*) were assayed with antibodies reacting against different garlic-infecting viruses. *1* Anti-OYDV-O (Mayoral); *2* anti-OYDV-O (Graichen); *3* anti-GCLV (Graichen); *4* anti-OYDV-O (Maat); *5* anti-LYSV (Lot); *6* anti-SLV (Maat). Cuts *1*, *2–3* and *4–6* were obtained from different gels. The open arrowhead indicates the 20 kDa polypeptide position, putatively derived by proteolysis of GP1. The black arrowhead indicates the position of the 30 kDa GP1 polypetide

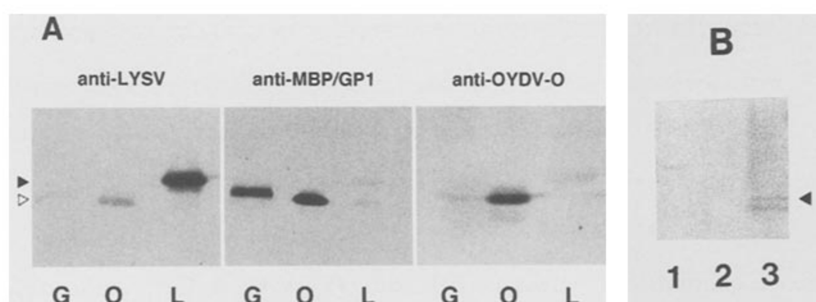

Fig. 3. Western blot analysis of purified virions and extracts from virus-infected plants. **A** *G* Partially purified OYDV-G virions; *O* partially purified OYDV-O virions; *L* partially purified LYSV virions. The antibody employed in each assay is indicated aboved the respective panel. Anti-LYSV and anti-OYDV-O antibodies were from Lot and Graichen, respectively. **B** Extract from healthy garlic (*1*), LYSV-infected leek (*2*) and OYDV-O-infected garlic (*3*) plants assayed with anti-MBP/GP1 antiserum. The arrowhead indicates the position corresponding to the OYDV coat protein

fragment corresponding to the size expected for the CP gene and the 3′ non-coding region was thus obtained. After cloning and sequencing of this fragment, a partial amino acid sequence for the OYDV-O CP was deduced. Comparison with the analogous sequence of GP1 showed a similarity of 88% (Fig. 4A). Most of the amino acid changes were concentrated in the N-terminal region. Remarkably, similarity between the 3′ non-translated regions of the GP1 and OYDV-O genomes is 82.9% (Fig. 4B).

A

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GP1      QAGDGEDAAAQSNTSKQVSKQNHKDVDAGTTGKFTVPRIKALSDKMRFPKVGKSVVLNAD
                      : ::  ::  ::::::::: : :::::::::::::::::: :::::
OYDV-O   .............TTKQIAKQKDKDVDAGTTGQFSVPRIKALSDKMRFPKVGKNVVLNAE

GP1      DLLAYKPDQIELYNTRATQQQFENWFGAIKKEYDVNDEQMKIILNGLMVWCIENGTSPNL
          :: ::: ::::::::::  :::::  :::::::: ::::::::::::::::::::::::
OYDV-O   HLLTYKPEQIELYNTRATLRQFENWYMAIKKEYDVDDEQMKIILNGLMVWCIENGTSPNL

GP1      SGNWTMMDGDEQVEYPLAPILDNAKPTFRQIMAHFSDAAEAYIEYRNATEKYMPRYGLQR
          :::::::: ::::::::: ::::::::::::::::::::::::::::::::::::::::
OYDV-O   TGNWTMMDGEEQVEYPLAPVLDNAKPTFRQIMAHFSDAAEAYIEYRNATEKYMPRYGLQR

GP1      NLTELSLARYAFDFYEMTSKTPKRAKEAHMQMKAAAVRGATNRLFGLDGNVNTTEEDTER
         :::::::::::::::::::::: :::::::::::: :::: :: :::::::::: :::::
OYDV-O   NLTELSLARYAFDFYEMTSKTPTRAKEAHMQMKAARVRGAANRSFGLDGNVNTTREDTER

GP1      HTAADVNKNQHTLLGIRM*
         ::: :::: :::::: :::
OYDV-O   HTATDVNKHQHTLLGVRM*
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B

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GP1      ACGCCAGTTGTATGTCTTTAGTTTTATATGTACCTTATATAAAAACGCACTTAGTATGT
         |  |||||||||||| |||| |   ||||  |  |||||||||||||||| ||||||||
OYDV-O   AAACCAGTTGTATGTTTTTA-T---ATAT--A--TTATATAAAAACGCACCTAGTATGT

GP1      ATTCCTCTCT-ACTTCGTTTAACCTGACTTCGAGCAGCGAAATGTGGTGTACCACTAGT
         |||||| | | | ||||||||||||||||| ||||| ||||| |||||||||||| |||
OYDV-O   ATTCCTTT-TCATTTCGTTTAACCTGACTTTGAGCAACGAAACGTGGTGTACCACCAGT

GP1      TGTGGCTGAACGATACGGTTAAATATCTGTGATTAATGTCGGGCTTCATGAGAGTGACG
         ||||||  ||||| | ||||||| || |||||||||||||| |||||||||| |||| |
OYDV-O   TGTGGCA-AACGAAAAGGTTAAACATTTGTGATTAATGTCGAGCTTCATGAGGGTGATG

GP1      TATCACGAGGTGACTCTTGGCGTGAATTACAGAGACAAAAAAAA
         ||||||| | ||| | |||||||||||||| || | ||||||
OYDV-O   TATCACG-GATGAATGTTGGCGTGAATTAC-GA-AGAAAAAA
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Fig. 4. Sequence comparisons between GP1 and OYDV-O. **A** GP1 garlic isolate and OYDV-O CP sequence comparison. The amino acids located at N-terminus of the OYDV-O CP could not be determined. Homologous amino acids are marked by colons. **B** Nucleotide sequences comparison between the GP1 garlic isolate and the OYDV-O 3′ non-coding regions. The first nucleotide from each sequence corresponds to that next to the polyprotein stop codon. Optimal alignment of both sequences was obtained with the DNAsis Maxim Homology (Hitachi Software, Japan) sequence analysis program

Discussion

A cDNA library representing several garlic viruses was synthesised using a viral RNA preparation obtained from infected garlic plants. Partial sequences from an unknown potyvirus (provisionally termed GP1) and from a recently described mite-borne garlic virus were obtained from this library. In addition, the coat protein gene of an onion isolate of OYDV (OYDV-O) was cloned and sequenced. On the basis of three different lines of evidence, we concluded that the GP1 sequence belongs to OYDV-G.

Firstly, we compared the CP sequence deduced for GP1 with that obtained for OYDV-O and found a similarity of 88%. It has been previously shown that, while the percentages of similarity between the CPs sequences of distinct potyviruses range from 38 to 71%, those between different viral strains vary between 90 and 98% [28]. Therefore, it was proposed that the degree of similarity between the CP sequences could be employed as a useful taxonomic criterion to differentiate members from viral strains in this important group [28]. The degree of similarity between the GP1 and OYDV-O CPs strongly suggests that these isolates represent different strains of the same virus, rather than different viral species. The high nucleotide sequence similarity found between the 3′ non-translated regions (82.9%) further supports this conclusion [16]. Secondly, the GP1 CP produced in *E. coli* cells was specifically recognised by polyclonal antibodies produced against OYDV, while it did not react with antibodies raised against other common garlic-infecting viruses. In addition, Western blot assays showed that the GP1 polypeptide has the same electrophoretic mobility as the OYDV-G CP obtained from infected garlic. This mobility is also in agreement with the MW deduced from the GP1 CP sequence (about 30 kDa). Thirdly, a fusion protein containing the GP1 sequence was expressed in bacteria and used as antigen to obtain polyclonal antibodies. These antibodies strongly reacted with the OYDV CP in assays performed with virion preparations, but showed a rather low affinity when tested against OYDV-infected leaf extracts. Besides, this antiserum exhibits a considerable degree of cross-reaction against PVY and other potyviruses in IEM-D assays (data not shown). A probable reason for this is that it includes antibodies recognising the CP central region, which is highly homologous in all potyviruses [29]. Due to their low specificity, anti-MBP/GP1 antibodies are not suitable for utilisation in diagnostic procedures. One possible way to overcome this problem would be to produce antibodies against a protein fragment solely comprising the N-terminal region of the viral CP [29]. On the other hand, even though OYDV-G and OYDV-O CPs appear to have a slightly different MW, the close immunological relationship and the high amino acid sequence similarity found between both viral strains validates the usefulness of anti-OYDV-O antibodies for certification purposes in garlic plants.

To our best knowledge, this is the first report dealing with the garlic and onion strains of OYDV at the molecular level. Two genomic sequences including CP genes have been previously reported for garlic potyviruses [26]. While one of them was assigned to LYSV [37], the other sequence was not associated to any known garlic virus and is not related to that described here. More recently, a partial genomic sequence corresponding to an OYDV isolate from *A. fistulosum* was reported by Tsuneyoshi et al. (GeneBank accession No. D73378). The similarities between the deduced amino acid sequence of this isolate and the corresponding sequences of GP1 and OYDV-O (74 and 73%, respectively), strongly suggest the presence of different OYDV strains specifically infecting each *Allium* species [33]. This presumption is supported by previous studies showing that OYDV-G does not readily infect onion and, vice versa, that

OYDV-O cannot develop infections in garlic [10], but no data are currently available on the infection range of the *A. fistulosum* strain.

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