

Characterization of *Pepper yellow leaf curl virus*, a tentative new *Polerovirus* species causing a yellowing disease of pepper

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Abstract Pepper (*Capsicum annuum*) is an important crop worldwide. In Israel, approximately 2,500 ha are grown all year round for the local and export markets. Herein, we report the identification of a viral pathogen causing a new devastating disease in pepper crops. The disease syndrome includes shortening of stem internodes, interveinal yellowing, and upward rolling of the leaf blade, accompanied by fruit discoloration and size reduction. Virus purification from infected plants yielded isometric particles, 25 nm in diameter. The causal agent of the disease was tentatively named *Pepper yellow leaf curl virus* (PYLCV). The virus cross-reacted in DAS ELISA with antisera against *Cucurbit aphid-borne yellows virus* and *Potato leafroll virus*, members of the *Polerovirus* genus. The partial nucleotide sequence obtained from the cloned viral coat protein and movement protein genes indicated 92% identity at the amino acid level with *Tobacco vein distortion virus* (TVDV), another member of the *Polerovirus* genus. However, the host range of PYLCV is significantly different from the host range described for TVDV. Based on our findings, the taxonomic status of PYLCV is discussed.

Keywords *Luteoviridae* · *Myzus persicae* · Persistent transmission · *Tobacco vein distortion virus* (TVDV)

Introduction

A high incidence of yellow dwarfed pepper plants (*Capsicum annuum*) was observed during the autumn of 1998 in the Arava Valley of Israel. Infected plants were found in greenhouses as well as in open fields. Symptoms of diseased plants included shortening of the internodes, interveinal yellowing, narrowing of leaves and upward rolling of leaf margins. The fruits of diseased plants were smaller than normal and discolored, resulting in reduced commercial value. From 1998 onwards the disease has spread to the Jordan and Arava valleys and across the country.

Previous results (Antignus *et al.* 2001, 2003) have shown that the causal agent of this pepper yellowing disease is a virus that has morphological, biological and molecular similarities to those described for luteoviruses. The virus was tentatively named *Pepper yellow leaf curl virus* (PYLCV).

The family *Luteoviridae* is composed of three genera: *Enamovirus*, *Luteovirus* and *Polerovirus*. Luteoviruses and poleroviruses are transmitted exclusively by aphids in a persistent (circulative) and nonpropagative mode (Raccah and Fereres 2009). Serological relationships within a genus are closer than those found among genera in the family. All family members have a linear, positive sense ssRNA

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packed in isometric virions, 25–30 nm in size. The size of the viral nucleic acid is between 5600 and 5882 nts. The genomes contain five or six open reading frames (ORFs) and the differences among members of the three genera are located principally in the 5' end of the genome. The genera of the family can be distinguished on the basis of the arrangements and sizes of the ORFs. Another important characteristic of the *Luteoviridae* is the presence of two virion structural proteins: the coat protein (CP) and a 'read-through' protein, which is a fusion of the products of the CP and the adjacent ORF5. The read-through protein may be associated with aphid transmission or virion stability (D'Arcy *et al.* 2000; Miller 1999). The present study was directed to identify and characterize the virus that causes the devastating yellowing disease of pepper crops in Israel.

Materials and methods

Virus purification and antiserum preparation The virus was isolated from infected pepper leaves. Fresh material was found to be a better source for purification than frozen material. Purification was carried out essentially as described by Rowhani and Stace-Smith (1979). Virus yield was calculated using an extinction coefficient ($A_1^{0.1\%}_{\text{cm},260\text{ nm}}=7$, determined for *Carrot red leaf virus* (Waterhouse and Murrant 1981). Samples containing the purified viral particles were stained with 1% uranyl acetate before being analyzed by transmission electron microscopy (TEM) (Tecnai G², FEI-Philips, Eindhoven, the Netherlands).

Virus transmission by insects The green peach aphid *Myzus persicae* (Sulzer) was raised on mustard (*Brassica perviridis* cv. 'Tendergreen'). Aphids were allowed a 48-h acquisition access feeding (AAF). Groups of ten to 20 individuals were then caged on the lower leaf surface of healthy pepper test plants at the three-leaf stage. Insects were given an inoculation access feeding (IAF) of 48 h before being treated with the insecticide imidacloprid (Confidor, Bayer CropScience Ltd., Australia) and transferred to a growth chamber for symptom development.

Characterization of the viral coat protein The molecular weight (MW) of the viral CP subunit was determined by SDS-PAGE of purified virions. Elec-

trophoresis was carried out in 10% acrylamide gels containing 0.4% sodium dodecylsulfate (SDS), according to Laemmli (1970). The resulting protein bands were visualized by staining with Coomassie Brilliant Blue (Sigma-Aldrich Corp., St. Louis, MO, USA) or excised from gel for further analysis.

Serological assays Antiserum against PYLCV was prepared using a purified virus preparation (0.5 mg ml⁻¹). The antigen was mixed with an equal volume of Freund's incomplete adjuvant and injected intramuscularly three times at monthly intervals. The rabbit was bled 7 days after the last injection.

Antiserum kits against *Potato leafroll virus* (PLRV) and *Beet western yellows virus* (BWYV) were obtained from Loewe Phytodiagnostica Biochemica GmbH (Sauerlach, Germany). Antiserum against *Cucurbit aphid-borne yellows virus* (CABYV) was kindly provided by Dr. Herve Lecoq (INRA, Avignon, France). Enzyme linked immunosorbent assays (ELISA) were performed as described by Clark and Adams (1977). Indirect ELISA on plates not coated with antibodies was done as described by Koenig (1981). Wells were coated with purified virus preparations for 4 h at 37°C. The antiserum was incubated for 1.5 h at 37°C or overnight at 4°C, followed by conjugated goat anti-rabbit globulin diluted 1:2000 and incubated for 3.5 h at 37°C. Substrate hydrolysis time was 20–40 min. Plates were recorded at 405 nm by an ELISA reader (Anthos, Salzburg, Austria). For Western blot analysis, proteins were blotted onto nitrocellulose membranes with a Mini Trans Blot Device (Bio-Rad, Richmond, VA, USA). For staining, the antiserum of CABYV was diluted 1:1000 while the homologous antiserum against PYLCV was used at a 1:500 dilution. Immunostaining was carried out as described by Hibi and Saito (1985), with the homologous antiserum used as first antibody at a dilution of 1:500.

RNA extraction Purified virus preparations served as a source for virion RNA extraction as described previously (Rosner *et al.* 1983). In general, purified virus preparations were incubated with RQ RNase free DNase I (Promega, Madison, WI, USA) for 1 h at 37°C followed by Proteinase K treatments at a final concentration of 200 µg ml⁻¹. The reaction mixture was incubated for 1 h at 37°C. Then the viral nucleic acids were purified by extraction with acidic phenol

chloroform (Ambion/Applied Biosystems, Austin, TX, USA). The aqueous phase of the preparation was precipitated overnight in the presence of glycogen (Fermentas, Vilnius, Lithuania), 0.1 M Na acetate and isopropanol at -20°C . The precipitated viral RNA was washed twice with 75% ethanol and allowed to air-dry for 10 min. The dry viral RNA was kept at -20°C before use.

Analysis of the viral CP by liquid chromatography mass spectrometry (LC-MS) Mass spectrometry analysis of the amino acid sequence of the viral CP was determined by LC-MS analysis by the Laboratory Interdepartmental Equipment Unit of The Hebrew University Medical School. A sample of the 27 kDa fraction corresponding to the virus CP (excised from acrylamide gel) was digested with trypsin; then the peptide mixture was solid-phase-extracted with the C18 resin-filled tip (ZipTip Milipore, Billerica, MA, USA) and nanosprayed into the Orbi-trap MS system in 50% CH_3CN 1% CHOOH solution. Mass spec-

trometry was carried out with the Orbitrap (Thermo Finnigan, Fisher Scientific, San Jose, CA, USA) using a nanospray attachment. Data analysis was done using the BioWorks 3.3 package (Thermo Electron) and database searches were performed against the NCBI nr database with the Mascot package (Matrix Science, London, UK). The obtained LC-MS peptides: [R.SSNSETFIFNK.D; K.LTSLQSTLR.K; K.NVAAGFFQIR.Y; R.YTVQLHNP.K] were showing a high sequence identity to peptides related to Chinese isolates of *Tobacco vein distorting virus* (TVDV) (accession no. AM411003 and EF529624). The four peptides obtained by the LC-MS analysis covered about 20% of the total TVDV CP amino acids sequence.

Primers design The amino acid sequences derived from the LC-MS analysis served to design degenerate primers by reverse prediction for polymerase chain reaction (PCR) amplification. A forward primer F1-PYLCV: '5 GGAGGAAGGTCRAGCAACAGC '3 (position 3824-3844 of the TVDV sequence) and a

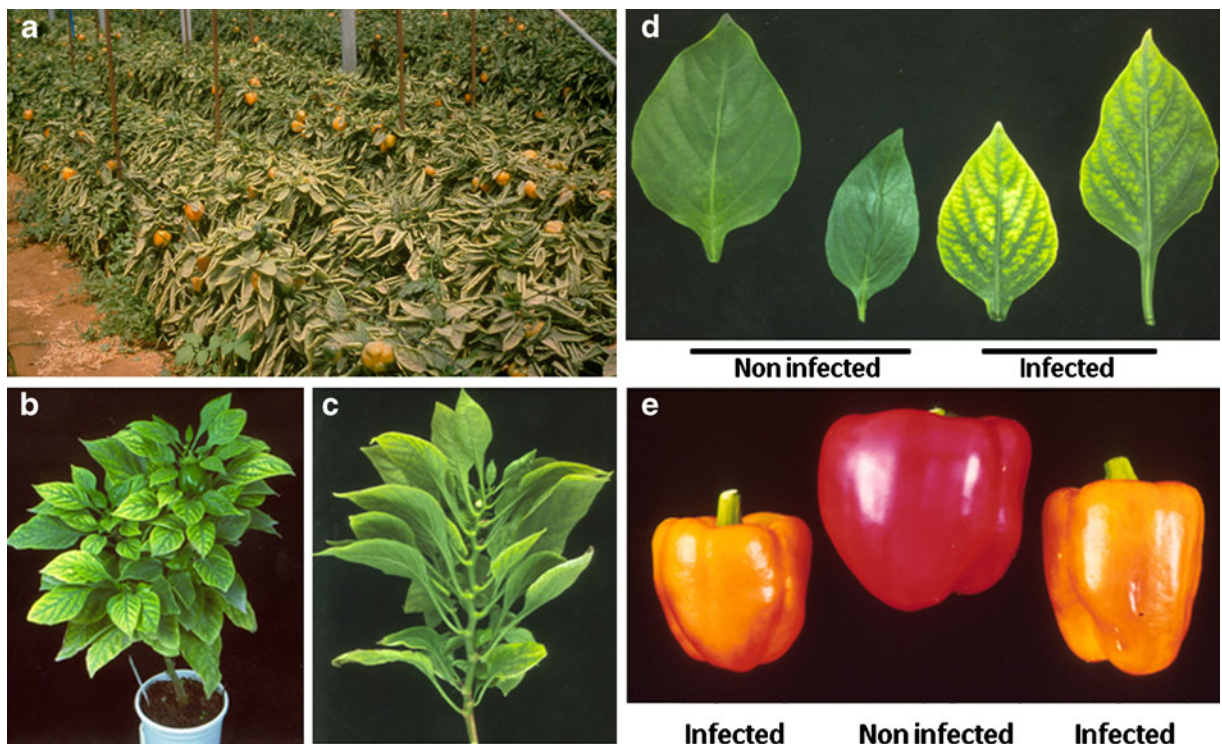


Fig. 1 Symptoms induced by *Pepper yellow leaf curl virus* (PYLCV). **a** High disease incidence in pepper grown in a commercial screenhouse in the Arava valley of Israel; **b–d** Interveinal yellowing and internode shortening in pepper plants

inoculated in the laboratory *via* aphids; **e** Pepper fruits collected from virus-infected plants, showing severe discoloration and size reduction

complementary primer: R2-PYLCV '5 CTATTTGG GGTGTGYARTTGCAC '3 (position 4209-4233 of the TVDV sequence) were synthesized.

RT PCR amplification The purified viral RNA served as a template for a reverse transcription (RT) reaction using a Verso™ cDNA Kit (Thermo Fisher Scientific, Epsom, UK) with the PYLCV complementary primer. The cDNA was amplified in PCR reaction using Taq polymerase (DreamTaq™, Fermentas) with specific and degenerated forward and reverse primers.

Cloning and sequence analysis PCR products were cloned into a pGEM-T-easy vector (Promega) according to the manufacturer's instructions. DNA plasmids were extracted with a plasmid extraction kit (Promega). Nucleotide sequencing was done on both strands, for each of the cloned genes by the Sequencing Service of the Molecular Biology Center (HyLab, Rehovot, Israel). Nucleotide sequence analysis was carried out using DNAMAN (Lynnon BioSoft, Montreal, Canada) and software from the National Center for Biotechnology Information (NCBI). Sequence homology was compared using

the Basic Local Alignment Search Tools (BLAST) <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. Multiple sequence alignments were analyzed and aligned with BioEdit and ClustalX softwares. Phylogenetic tree prediction was done using the TreeTop software http://www.genebee.msu.edu/services/phtree_reduced.html. The partial nucleotide sequences of the CP and the movement protein genes of PYLCV were deposited in the GenBank (accession no. HM439608).

Preparation of a radioactive-labeled riboprobe - Clones of PLRV CP gene in pUC 18 and those obtained from the PYLCV genome were linearized for *in vitro* transcription by *Sall* or *Apal* digestions. Transcription reactions were carried out in 200 mM Tris-HCl containing 30 mM MgCl₂, 10 mM spermidine, 50 mM NaCl and in the presence of [α -³²P] UTP 3000 Ci /mmole (Amersham Pharmacia Biotech, Buckinghamshire, UK). rNTPs were used at a final concentration of 0.5 mM (100 μ M UTP).

Dot spot hybridization PYLCV RNA preparations were denatured by mixing with an equal volume of 8XSSC, 10% formaldehyde, 5% formamide and

Table 1 Host range and description of symptoms induced by *Pepper yellow leaf curl virus* (PYLCV). Plants were inoculated via aphids (*Myzus persicae*). Virus infection was confirmed by both ELISA and two riboprobes prepared from the coat protein genes of *Potato leaf curl virus* (PLRV) and *Pepper yellow leaf curl virus* (PYLCV)

Host plant	Symptoms	PYLCV infection
<i>Capsicum annuum</i> cv. Maor	Yellowing, leaf curling, narrowing of leaves, spreading systematically	+
<i>Chenopodium amaranticolor</i>	Yellowing, small local lesions	+
<i>Cucumis sativus</i> cv. Beit Alpha	No symptoms	-
<i>Cucumis melo</i> cv. Arava	No symptoms	NT
<i>Citrullus vulgaris</i> cv. Malali	No symptoms	NT
<i>Cucurbita pepo</i> cv. Mayan	Yellowing	+
<i>Datura stramonium</i>	Mild interveinal yellowing	+
<i>Gomphrena globosa</i>	No symptoms	+
<i>Lycopersicon esculentum</i>	No symptoms	NT
<i>Nicotiana clevelandii</i>	Yellowing	+
<i>Nicotiana benthamiana</i>	No symptoms	+
<i>Nicotiana glutinosa</i>	No symptoms	-
<i>Nicotiana tabacum</i> cv. Samsun	No symptoms	-
<i>Nicotiana tabacum</i> cv. Samsun NN	No symptoms	NT
<i>Nicotiana tabacum</i> cv. Xanthi	No symptoms	NT
<i>Nicotiana sylvestris</i>	No symptoms	-
<i>Nicotiana rustica</i>	No symptoms	-
<i>Petunia hybrida</i>	Yellowing	+
<i>Physalis floridana</i>	Yellowing small leaves	+

+ infected, - not infected, NT not tested by ELISA or a riboprobe

incubation at 65°C for 15 min. Five μ l of the denatured viral RNA was spotted on a BioRad nylon membrane (BioRad, Hercules, CA, USA) and baked at 80°C for 2 h. The hybridization was performed with riboprobes prepared from the above mentioned viral c-DNA clones. Signals were recorded by membrane exposure in a Bio-Imaging Analyzer, Bas 1500 IP Reader (Fujifilm, Tokyo, Japan).

DIG labeling and hybridization A DNA fragment derived from the CP gene of PYLCV was cloned in pGEM-T-easy vector and was used for probe preparation. DIG (digoxigenin)-labeled nucleotide was introduced into the probe by PCR. The reaction mixture had a final volume of 50 μ l and contained

the following components: 1X of Dream Taq Polymerase buffer (100 mM Tris-HCl pH 8.0, 500 mM KCl pH 8.3, and 25 mM MgCl₂), 100 pM of the forward primer F1-PYLCV, the complementary primer R2-PYLCV, dNTP's mixture containing DIG-labeled dUTP (2 mM dATP, dCTP, dGTP, 1.3 mM dTTP, and 0.7 mM alkali labile DIG-11 dUTP), 100 pM DNA template and 1.5 Unit of DreamTaq polymerase. The PCR reaction was programmed for 35 cycles under the following conditions: a DNA denaturation step at 94°C for 30 s, a primer annealing step for 30 s at 60°C and a 30 s primer extension step at 72°C. Two μ l of each sample was blotted on a positive charged membrane (Roche, Basel, Switzerland) and then cross-linked under UV for 3 min.

Fig. 2 Characterization of *Pepper yellow leaf curl virus* (PYLCV): **a** Electron micrograph of purified particles with modal size of 25–27 nm (bar represents 100 nm); **b** Electrophoretic separation of PYLCV viral RNA on 1% Tris-Borate-EDTA (TBE) agarose gel stained with ethidium bromide; **c** Cross-hybridization of the coat protein (CP) gene of *Potato leaf curl virus* (PLRV) and PYLCV genomic ribonucleic acid (RNA) by dot spot hybridization analysis. A riboprobe was prepared from the cloned CP gene of PLRV. 1. Cloned PLRV CP gene. 2. Genomic RNA of an unrelated tobamovirus: *Tomato mosaic virus* (ToMV). 3. Denatured purified virions of PYLCV. 4. PYLCV genomic RNA from purified virions. **d** specific hybridization of the CP gene of PYLCV in dot spot hybridization analysis. A DIG probe was prepared from the cloned CP gene of PYLCV: 1. PYLCV virions. 2. PYLCV genomic RNA from purified virions. 3. ToMV virions. 4. ToMV genomic RNA from purified virions. 5. Total RNA extracted from healthy pepper plant

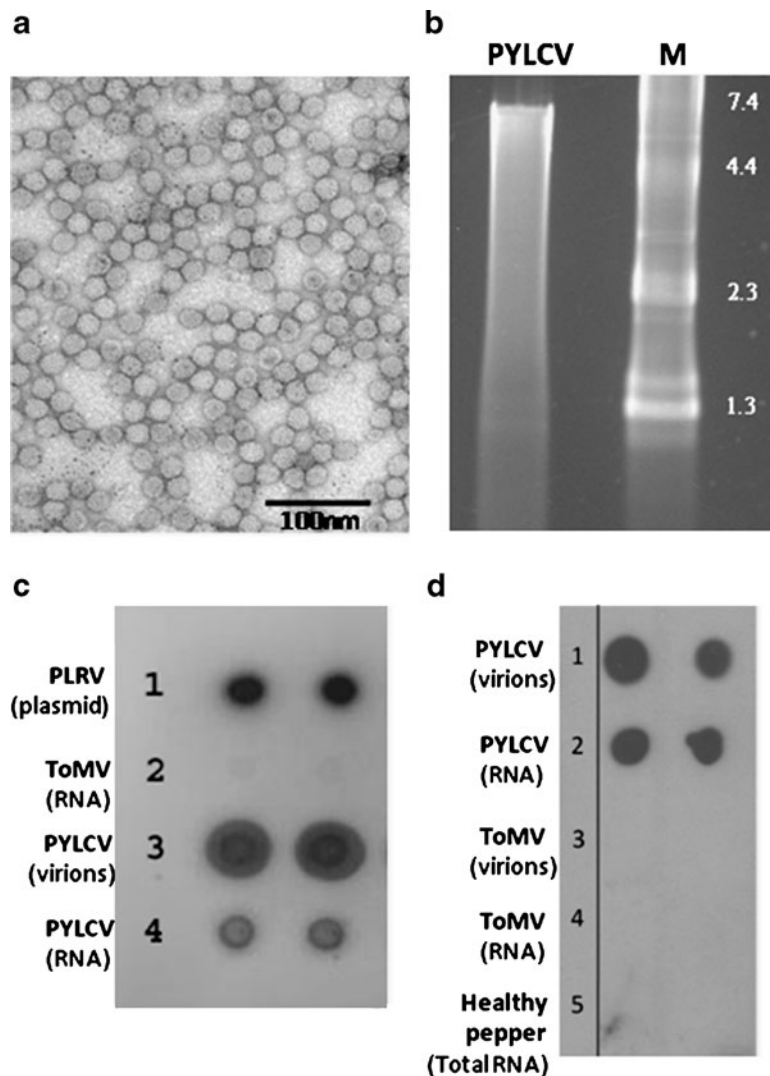
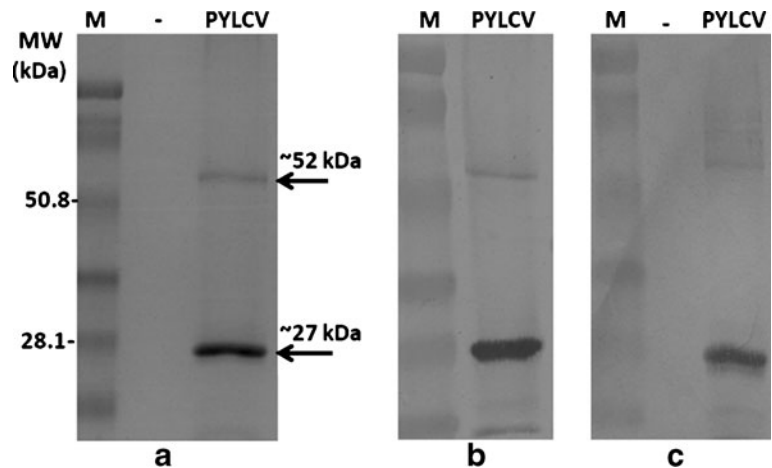


Fig. 3 PAGE separation of the coat protein subunit of *Pepper yellow leaf curl virus* (PYLCV) in the presence of 10% sodium dodecyl sulfate (SDS). *Left*: gel stained with Coomassie brilliant blue; *Middle*: Western blots analysis using PYLCV homologous antiserum; *Right*: antiserum against *Cucurbit aphid-borne yellows virus* (CABYV). *M* represents the protein marker ladder



Prehybridization was carried out at 50°C in DIG Easy Hyb solution (Roche). Hybridization was performed at 50°C overnight. Then the membrane was washed at high stringency. A chemofluorescent kit CSPD® (Roche Molecular Biochemicals) was applied to detect the probe signal on X-ray film.

Results

Symptomatology and host range Disease syndrome in infected pepper plants included severe leaf interveinal yellowing (Fig. 1b, d), significant shortening of stem internodes, leading to plant stunting (Fig. 1a, c), upward rolling of leaf blades, with increase in leaf

brittleness (Fig. 1a), fruit discoloration and size reduction (Fig. 1e). Table 1 demonstrates the partial laboratory host range of PYLCV obtained from inoculation tests using *Myzus persicae* as a vector. As shown, plants belonging to the *Solanaceae*, *Cucurbitaceae*, *Chenopodiaceae*, *Apocynaceae* and *Verbenaceae* were infected by the virus.

Virus transmission All attempts to transmit PYLCV by mechanical inoculation failed; however, the virus was successfully transmitted to healthy plants by grafting (not shown). Field observations have indicated an overlapping of virus-infected pepper plants foci and foci of aphid-infested plants. Virus-free aphids (*M. persicae*) served for laboratory transmission experiments. In several independent experiments the

Table 2 Indirect ELISA reactions of purified preparation of *Pepper yellow leaf curl virus* (PYLCV) antigen with antisera of different poleroviruses, demonstrating serological cross reaction with members of the *Polerovirus* genus

	Dilution factor of PYLCV antigen (mg ml ⁻¹)			
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
Antiserum type	^z ELISA value (A ₄₀₅)			
As α PLRV	0.530	0.272	0.225	0.087
As α BWYV	0.592	0.239	0.201	0.096
As α CABYV	2.706	0.102	0.001	0.001

PLRV Potato leafroll virus, *BWYV* Beet western yellows virus, *CABYV* Cucurbit aphid-borne yellows virus

^zThe nonspecific background for these reactions was 0.09 as estimated by using a purified preparation of the unrelated *Tomato mosaic virus* (ToMV)

Table 3 DAS ELISA reactions of *Pepper yellow leaf curl virus* (PYLCV) purified antigen with its homologous antiserum and with antiserum against *Cucurbit aphid-borne yellows virus* (CABYV)

	Dilution factor of PYLCV antigen (mg ml ⁻¹)			
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴
Antiserum type	ELISA value (A ₄₀₅)			
As α PYLCV ^z	0.957	0.600	0.452	0.073
As α CABYV ^y	0.156	0.118	0.097	0.052

^zThe nonspecific background for PYLCV antiserum was 0.015 when purified preparation of the unrelated *Tobamovirus Cucurbit fruit mottle mosaic virus* (CFMMV) served as a control

^yThe nonspecific background for *Cucurbit aphid-borne yellows virus* antiserum was 0.00 when purified CFMMV served as a control

virus was successfully transmitted from infected pepper plant to healthy ones, by the procedure described in “Materials and methods”.

Transmission efficiency reached 95% when groups of ten aphids per plant were used. Successful transmission was obtained in another set of experiments where aphids (*Aphis gossypii*) that colonized infected pepper plants in the field, were transferred to healthy pepper test plants. A minimum value of 7 days was determined for the virus retention time in *M. persicae*. This parameter was determined by serial transmission of viruliferous aphids to healthy pepper plants at one-day intervals between each transfer (not shown).

Virus purification Electromagnetic (EM) analysis of virus-purified preparations demonstrated the presence of isometric virus particles, 25 nm in size and with slightly hexagonal morphology (Fig. 2a). Virus yield was approximately 100 µg per 300 g of infected pepper leaves.

Characterization of the viral coat protein Two structural proteins with molecular weights of 27 kDa and

52 kDa were identified when purified preparations of PYLCV were separated by 10% SDS-PAGE (Fig. 3a).

Characterization of the virus nucleic acid The viral ribonucleic acid (RNA) was extracted from purified virions and separated on Tris-borate-EDTA (TBE) gels. An RNA fraction with an estimated size of ~6 Kb could be detected on the gel (Fig. 2b).

Serological relationship Based on the above mentioned results, it was hypothesized that PYLCV is a tentative member of the *Luteoviridae*. This possibility was tested by reacting purified virus preparations with specific antisera of the following *Luteoviridae* members: PLRV, BWYV and CABYV. The results presented in Table 2 show that all the tested antisera reacted with the virus by Indirect ELISA (Table 2). On the other hand, PLRV and BWYV antisera failed to react when the more specific DAS-ELISA method was used instead. However, CABYV antiserum strongly reacted by this procedure, indicating a closer serological relationship with the studied virus (Table 3). The similarity in the immuno reaction between

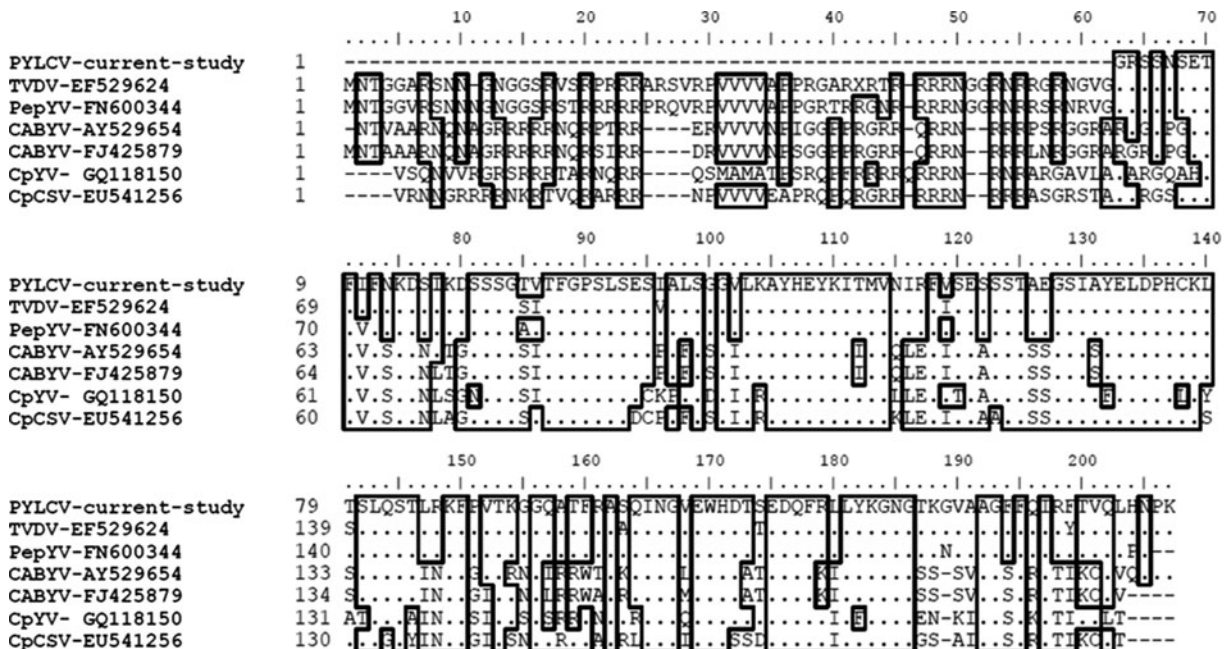


Fig. 4 Alignment of the deduced amino acids sequences of the coat proteins of six members of the *Luteoviridae* showing the highest BLAST similarity. Line 1: *Pepper yellow leaf curl virus* (PYLCV), the current study (accession no. HM439608); Line 2: *Tobacco vein distorting virus* (TVDV) (accession no. EF529624); Line 3: *Pepper yellowing virus* (PepYV) (acces-

sion no. FN600344); Line 4: *Cucurbit aphid-borne yellows virus* (CABYV) (accession nos. AY529654 and FJ425879); Line 5: *Chickpea yellows virus* (CpYV) (accession no. GQ118150); Line 6: *Chickpea chlorotic stunt virus* (CpCSV) (accession no. EU541256)

PYLCV and CABYV is further demonstrated in Fig. 3, which shows that both the 27 kDa and 52 kDa fractions of the viral CP react with CABYV antiserum. However, the signal level in this case was significantly lower than that obtained with the PYLCV homologous antiserum (Fig. 3).

Genome relationships Dot-blot hybridization assays served to evaluate genomic identities between PYLCV and the polerovirus PLRV. Radioactive-labeled riboprobes, prepared from the cloned CP gene of PLRV, reacted strongly in dot spot hybridization with denatured virus particles or free RNA extracted from purified virions. The probe failed to react with a purified preparation of the unrelated tobamovirus *Tomato mosaic virus* (ToMV) (Fig. 2c).

Amplification and cloning of viral genes PYLCV RNA was used for RT reactions using random hexamer primers. Then the cDNA was amplified by PCR reaction using Taq polymerase in the presence of

degenerate primers, designed on the basis of the aa sequence derived from the LC-MS analysis (see “Materials and methods”). The reaction yielded a DNA fragment of 438 bp which was cloned in pGEM-T- easy plasmid.

To confirm the viral origin of the clone, a DIG probe was prepared from the cloned viral DNA and used in hybridization experiments as described in “Materials and methods”. As shown in Fig. 2c and d, the probe reacted specifically with purified virions as well as with purified viral RNA but failed to react with the genome of an unrelated virus (ToMV) or total RNA from a healthy pepper plant.

BLAST search, sequence analysis of the 438 bp PCR product revealed high nucleotide sequence identity with viruses of the *Polerovirus* genus: 92% identity was found with the Chinese isolate of TVDV and 79% for the Chinese isolate of the polerovirus CABYV (accession no. FJ425879). Identity of 79% was calculated for the Ethiopian polerovirus *Chickpea chlorotic stunt virus* (CpCSV) and 78% with the

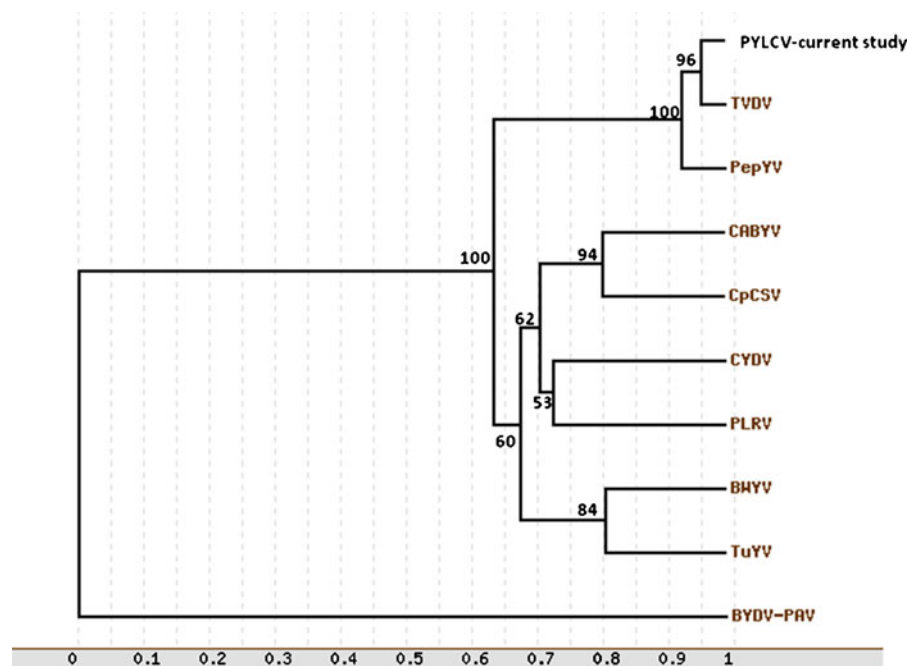


Fig. 5 A phylogenetic tree constructed on the basis of partial amino acid sequences of the CP gene of representative members of the *Luteoviridae*: *Pepper yellow leaf curl virus* (PYLCV), the current study (accession no. HM439608); *Tobacco vein distorting virus* (TVDV) (accession no. EF529624); *Pepper yellowing virus* (PepYV) (accession no. FN600344); *Cucurbit aphid-borne yellows virus* (CABYV) (accession no. FJ425879); *Chickpea yellows virus* (CpYV)

(accession no. GQ118150); *Chickpea chlorotic stunt virus* (CpCSV) (accession no. EU541256); *Beet western yellows virus* (BWYV) (accession no. AAB00348); *Turnip yellows virus* (TuYV) (accession no. ADE93576); *Cereal yellow dwarf virus* (CYDV) (accession no. ABP68747); *Potato leafroll virus* (PLRV) (accession no. AAA46870); *Barley yellow dwarf virus-PAV* (BYDV-PAV) (accession no. FJ687413)

Sudanese *Chickpea yellows virus* (accession no. GQ118150), an unassigned luteovirus. Similar identity values were obtained following BLAST search analysis at the amino acids level (not shown).

BLAST hit results alignment was performed for the 145aa sequence of the viral CP (Fig. 4). A phylogenetic tree was constructed on the basis of the partial PYLCV CP sequence and homologous sequences of other members of the *Luteoviridae* (Fig. 5). The phylogenetic relationships of the compared viruses fall into three separate clads: The outgrouping clad represented by BYDV-PAV, a clad of closely related poleroviruses divided into three subgroups, and a third clad of the closely related PYLCV, TVDV and PepYV (Fig. 5).

Discussion

Of the 13 virus species of the genus *Polerovirus* [2009 release of the International Committee on Taxonomy of Viruses (ICTV)] (<http://www.ictvonline.org/virusTaxonomy.asp?bhcp=1>), only BWYV was reported to be able to infect pepper plants (Duffus 1960). However, the host range found for PYLCV is significantly different from the reported host range of BWYV and other members of the genus *Polerovirus* (Lecoq *et al.* 1992; Rochow *et al.* 1987; Smith 1991; Thomas 1987). Like other poleroviruses, PYLCV induces yellowing symptoms on its host plants (Fig. 1; Table 1). Field observations indicated that PYLCV-infected pepper plants grown in shaded parts of the greenhouse did not exhibit yellowing symptoms. Similar effects were reported previously for other luteoviruses and poleroviruses, indicating that high light intensity is critical for the expression of the yellowing symptoms (Rochow and Duffus 1981). Another characteristic symptom that PYLCV shares with other luteoviruses and poleroviruses is plant stunting (Fig. 1b). The origin of the PYLCV reservoir in nature is still unknown; however, *Datura stramonium*, which was identified as a host for PYLCV (Table 1), is a common weed in Israel and might play a role in virus epidemiology. Moreover, PYLCV is transmitted efficiently by at least two aphid vectors, *M. persicae* and *A. gossypii*, which are well known for their wide host range. The morphology of PYLCV particles (Fig. 2) is identical to the icosahedral particles described for the *Luteoviridae* members

(Miller 1999). The major CP component of PYLCV is approximately 27 kDa in size (Fig. 3), which is significantly higher than the reported MW of the capsid proteins of this family, which ranges between 21 kD and 23 kD (D'Arcy *et al.* 2000). The second component of PYLCV CP has an estimated MW of ~52 kD (Fig. 3), which is similar in size to the homologous proteins of the *Luteoviridae* members (D'Arcy *et al.* 2000). Serologically, PYLCV is cross-reacting with PLRV, BWYV and CABYV antisera when tested by the broad range indirect ELISA (Table 2); however, PLRV and BWYV failed to react with these antisera in the highly specific DAS-ELISA. These results indicate distant serological relationships among PYLCV, PLRV and BWYV. On the other hand, CABYV antiserum reacted with PYLCV antigen in DAS ELISA, demonstrating a closer serological relationship (Table 2). Based on particle morphology, symptomatology, mode of transmission, size of the viral genome, characteristics of the structural genes and serological relationships, it may be concluded that PYLCV is a tentative member of the *Polerovirus* genus of the *Luteoviridae* family. The sequencing data obtained from the cloned fragment of PYLCV CP and the viral MP genes show 92% nucleotide sequence identity (Figs. 4 and 5) with *Tobacco vein distorting virus* (TVDV), a member of the genus *Polerovirus*. TVDV was first reported in *Nicotiana tabacum* from Zimbabwe, where it spreads causing malformation of leaf veins in tobacco (Smith 1946). Mixed infections of TVDV and *Tobacco mottle virus* led to a syndrome named tobacco rosette disease (Cole 1962; Gates 1962; Smith 1946). TVDV acts as an assistant virus, enabling vector transmission of tobacco mottle virus (Smith 1946). According to the criteria used to demarcate species of the family *Luteoviridae*, a difference of more than 10% in the amino acid sequence of any gene product is required to differentiate among distinct virus species (D'Arcy *et al.* 2000). Ninety-two percent identities with TVDV suggest that PYLCV is a strain of TVDV, but the host range of the two viruses differs significantly. None of the plants that were reported as hosts of TVDV (*N. glutinosa*, *N. sylvestris*, *N. tabacum*) was infected by PYLCV when inoculated *via* aphids (Table 1). Moreover, *D. stramonium*, which is a common host for both viruses, reacts to TVDV by leaf vein malformation, whereas it reacts by interveinal yellowing to infection with PYLCV. These major differences in host range suggest that TVDV and

PYLCV are distinct entities. Recent studies have demonstrated the ability of *Luteoviridae* members to make intra- and interspecific recombination, especially at the boundaries of the RdRp and CP genes (Pagan and Holmes 2010; Smith *et al.* 2000). Based on that, it may be hypothesized that PYLCV is a product of recombination between TVDV and another polerovirus resulting in a modification of its biological properties. Therefore, the present level of knowledge does not allow an unambiguous decision on the taxonomic status of PYLCV and the solution for this dilemma must wait until the full sequence of PYLCV will be available.

PYLCV has a very high damage potential in pepper crops; however, anti-aphid insecticide (*e.g.* Confidor) treatments were found to be very efficient in preventing the virus from spreading by the aphid vectors.

References

- Antignus, Y., Lachman, O., Pearlsman, M., & Ucko, O. (2001). A new pepper yellowing disease of pepper caused by an unidentified *Luteovirus*. *Phytoparasitica*, *29*, 255 (abstr.).
- Antignus, Y., Lachman, O., Pearlsman, M., & Ucko, O. (2003). A new virus disease of pepper crops in Israel, caused by a luteovirus. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, *110*, 89 (abstr.).
- Clark, F., & Adams, A. N. (1977). Characterization of the microplate method of enzyme linked immunosorbent assay for detection of plant viruses. *Journal of General Virology*, *34*, 475–483.
- Cole, J. S. (1962). Isolation of tobacco vein distorting virus from tobacco plants infected with aphid-transmissible bushy-top. *Phytopathology*, *52*, 1312.
- D'Arcy, C. J., Domier, L. L., & Mayo, M. A. (2000). Family Luteoviridae. In M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estes, S. M. Lemon, et al. (Eds.), *Virus taxonomy, seventh report of the international committee of taxonomy of viruses* (pp. 775–784). San Diego, CA, USA: Academic.
- Duffus, J. E. (1960). Radish yellows, a disease of radish, sugar beet, and other crops. *Phytopathology*, *50*, 389–394.
- Gates, L. F. (1962). A virus causing axillary bud sprouting in tobacco in Rhodesia and Nyasaland. *Annals of Applied Biology*, *50*, 169–174.
- Hibi, T., & Saito, Y. (1985). A dot immunobinding assay for the detection of tobacco mosaic virus in infected tissues. *Journal of General Virology*, *66*, 1191–1194.
- Koenig, R. (1981). Indirect Elisa methods for the broad specificity detection of plant viruses. *Journal of General Virology*, *55*, 53–62.
- Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of head bacteriophage T4. *Nature, London*, *227*, 680–685.
- Lecoq, H., Bourdin, D., Wipf-Scheibel, C., Bon, M., Lot, H., Lemaire, O., et al. (1992). A new yellowing disease of cucurbits caused by a luteovirus, cucurbit aphid-borne yellows virus. *Plant Pathology*, *41*, 749–761.
- Miller, W. A. (1999). *Luteovirus (Luteoviridae)*. *Encyclopedia of virology*. London, UK: Academic. doi:10.1006/rwvi.1999.0170.
- Pagan, I., & Holmes, E. C. (2010). Long-term evolution of the Luteoviridae: time scale and mode of virus speciation. *Journal of Virology*, *84*, 6177–6187.
- Raccach, B., & Fereres, A. (2009). Insect transmission of plant viruses. *Encyclopedia of Life Sciences* (an on-line publication). doi:10.1002/9780470015902.A0021525.a0000760.pub2.
- Rochow, W. F., & Duffus, J. E. (1981). Luteoviruses and yellows diseases. In E. Krustak (Ed.), *Handbook of plant virus infections and comparative diagnosis* (pp. 147–170). Amsterdam, the Netherlands: Elsevier/North-Holland Biochemical.
- Rochow, W. F., Sward, R. J., & Waterhouse, P. M. (1987). Barley yellow dwarf luteovirus. In A. A. Brunt, K. Crabtree, M. J. Dallwitz, A. J. Gibbs, & L. Watson (Eds.), *Viruses of plants* (pp. 162–167). Wallingford, UK: CAB International.
- Rosner, A., Bar-Joseph, M., Moskovitz, M., & Mevarech, M. (1983). Diagnosis of specific viral RNA sequences in plant extracts by hybridization with polynucleotide kinase mediated ³²P-labelled, double stranded RNA probe. *Phytopathology*, *73*, 609–702.
- Rowhani, H., & Stace-Smith, R. (1979). Purification and characterization of *potato leafroll virus*. *Virology*, *98*, 45–54.
- Smith, G. R., Borg, Z., Lockhart, B. E. L., Braithwaite, K. S., & Gibbs, M. J. (2000). *Sugarcane yellow leaf virus*: a novel member of the Luteoviridae that probably arose by inter-species recombination. *Journal of General Virology*, *81*, 1865–1869.
- Smith, H. G. (1991). Beet mild yellowing luteovirus. In A. A. Brunt, K. Crabtree, M. J. Dallwitz, A. J. Gibbs, & L. Watson (Eds.), *Viruses of plants* (pp. 209–211). Wallingford, UK: CAB International.
- Smith, K. M. (1946). Tobacco rosette: a complex virus disease. *Parasitology*, *37*, 21–24.
- Thomas, J. E. (1987). Potato leafroll luteovirus. In A. A. Brunt, K. Crabtree, M. J. Dallwitz, A. J. Gibbs, & L. Watson (Eds.), *Viruses of plants* (pp. 1014–1018). Wallingford, UK: CAB International.
- Waterhouse, P. M., & Murant, A. F. (1981). Purification of *carrot red leaf virus* and evidence from four serological tests for its relationship to luteoviruses. *Journal of General Virology*, *57*, 191–204.