GRAPEVINE DEFORMATION VIRUS, A NOVEL NEPOVIRUS FROM TURKEY

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SUMMARY

A virus with isometric particles *ca*. 30 nm in diameter and angular contour was recovered by mechanical transmission from a grapevine with fanleaf-like symptoms growing at Nevsheir (Cappadocia, Turkey). In sucrose density gradient centrifugation this virus (laboratory code: isolate N66) sedimented as three components, T (empty shells), M, and B, both of which consisted of apparently intact particles. Virus preparations contained two RNA species with mol. wt. $2.6 \cdot 10^6$ Da (RNA-1) and $1.3 \cdot 10^6$ Da (RNA-2). The coat protein (CP) subunits were of a single type with M_r of *c.* 53,000. An antiserum with a titre of 1:1024 was raised, which did not react with healthy plant antigens. A fragment 1,175 nt in size from the 3' terminal region of RNA-2 was sequenced, which comprised part of the CP cistron. Comparison analysis with GenBank sequences from the same region revealed variable levels of homology with other grapevine nepoviruses, the closest being *Arabis mosaic virus* (ArMV, 69% identity at the amino acid level) and *Grapevine fanleaf virus* (GFLV, 58% identity at the amino acid level), both belonging in subgroup A of the genus *Nepovirus.* Based on the determined sequence, specific primers were designed, which in RT-PCR assays amplified a 240 bp fragment from grapevine crude tissue extracts. The physicochemical properties of isolate N66 and the cytopathology of infected *Chenopodium amaranticolor* tissues resembled very much those of nepoviruses. In gel double diffusion tests isolate N66 proved serologically unrelated to 16 different nepoviruses. A distant positive reaction was obtained with ArMV in immunodiffusion (serological differentiation index = 4) and immunoelectron microscopy tests and when leaf extracts from infected grapevines or *C. quinoa* were tested in ELISA with commercial antisera to ArMV. These results support the notion that isolate N66 is a hitherto undescribed virus species belonging in subgroup A of the genus *Nepovirus*, serologically related to but distinct from ArMV, for which the name Grapevine deformation virus (GDefV) is proposed.

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INTRODUCTION

In the course of a survey of virus diseases of the grapevine (*Vitis vinifera*) in Turkey (Cigsar *et al.*, 2002), a virus with isometric particles (laboratory code N66) was recovered by mechanical transmission from a vine of cv. Dimrit growing at Nevsheir (Cappadocia). The infected plant showed fanleaf-like symptoms i.e., variously distorted and mottled leaves with open petiolar sinuses, reduced lobes, asymmetric leaf blades (Fig. 1), irregular branching of the canes, short internodes, depressed growth, fewer and straggly clusters. The same symptoms were observed in rooted cuttings from the original mother plant that were grown in isolation under screehouse conditions at Bari (southern Italy).

In *Chenopodium amaranticolor*, N66 induced symptoms like those elicited by *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV) as described by Martelli (1993) and in gel double diffusion tests (GDT) it appeared to be serologically distantly related with ArMV but not with several other nepoviruses known to infect grapevines. An investigation was therefore carried out for determining some of the properties and the taxonomic position of this virus.

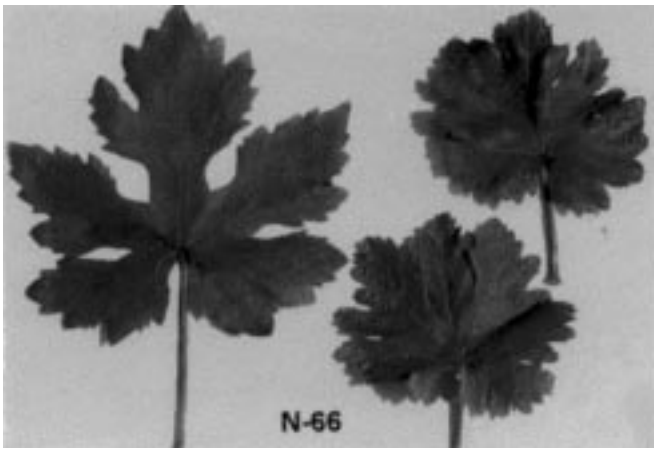

Fig. 1. Deformed leaves from a grapevine cv. Dimrit infected by isolate N66. Leaf from a non-infected vine on the left.

MATERIALS AND METHODS

Virus source and experimental host range. N66 isolation was from leaves of glasshouse-forced cuttings from the naturally infected cv. Dimrit vine. Leaf tissues were crushed in the presence of 0.1 M phosphate buffer, pH 7.2, containing 2.5% nicotine and the extract was rubbed onto celite-dusted leaves of a restricted range of herbaceous hosts. Virus cultures were maintained in *C. amaranticolor*, which was used as source of inoculum for transmission to other herbaceous hosts grown in a glasshouse at 22-24°C.

Virus purification. Virus extraction and purification was from *C. amaranticolor.* Systemically infected leaves were collected and homogenized in a blender with 2 vol. of cold 0.1 M phosphate buffer, pH 7.0, containing 0.1% thioglycolic acid. The extract was expressed through cheesecloth, clarified by stirring for 15 min in the presence of 5% Mg-activated bentonite (Dunn and Hitchborn, 1965) and centrifuged at 10,000 g for 15 min. Virus was precipitated with 10% (w/v) polyethylene glycol (MW 6,000) and 1% NaCl, under stirring at 4°C for 90 min. The precipitate was collected by low-speed centrifugation (15 min at 10,000 g), resuspended in phosphate buffer 0.02 M, pH 7.0, and centrifuged at 126,000 g for 90 min. Fractionation of concentrated partially purified virus preparations was in 10-40% linear sucrose density gradient columns centrifuged at 35,000 rpm for 2 h in a Beckman SW 41 rotor. Gradients were scanned at 254 nm with a ISCO ultraviolet absorbance monitor and the peaks corresponding to virus fractions were collected separately, dialyzed against 0.02 M phosphate buffer pH 7.2, and concentrated by high-speed centrifugation (90 min at 126,000 g).

Antiserum production and serology. An antiserum was raised in a New Zealand white rabbit, which was injected subcutaneously and intramuscularly with a purified virus preparation (*ca* 1 mg nucleoprotein) emulsified with an equal volume of Freund's incomplete adjuvant, followed by three intravenous injections at weekly intervals. Antiserum collection began one week after the last injection and its titre was determined by GDT (0.7% agar, 0.85% NaCl and 0.02% sodium azide) using partially purified virus. An ELISA kit was prepared by absorbing the crude antiserum with 2 vol. of healthy *C. amaranticolor* sap, incubating the mixture overnight at 4°C, and removing the precipitate by centrifugation at 5,000 g for 10 min. Globulins were purified by absorption onto a protein A-sepharose column as described by Clark and Bar-Joseph (1984), and aliquots of 1 mg ml^{-1} were conjugated with alkaline phosphatase by treating with 0.06% glutaraldehyde (Avrameas, 1969).

Serological relationships were investigated by GDT. N66 antiserum was allowed to react with concentrated partially purified extracts from herbaceous hosts infected with some grapevine-infecting nepoviruses, *i.e.* GFLV, ArMV, Strawberry latent ringspot virus (SLRSV), *Grapevine chrome mosaic virus* (GCMV), *Artichoke Italian latent virus* (AILV), *Grapevine Bulgarian latent virus* (GBLV), *Raspberry ringspot virus* (RpRSV), *Grapevine Tunisian ringspot virus* (GTRSV), *Tobacco ringspot virus* (TRSV), *Tomato blackring virus* (TBRV), *Tomato ringspot virus* (ToRSV), and Grapevine Anatolian ringspot virus (GARSV), a recently described nepovirus from south east Anatolia (Gokalp *et al.*, 2003). Furthermore, N66 antigen was tested against antisera to 17 different nepovirus species that included all of the above plus *Artichoke yellow ringspot virus* (AYRSV), *Blueberry leaf mottle virus* (BLMV), *Cherry leafroll virus* (CLRV), *Chicory yellow mottle virus* (ChYMV), *Myrobalan latent ringspot virus* (MLRSV), and *Peach rosette mosaic virus* (PRMV). The plates were left at room temperature and the precipitin bands observed after 24 h. Serological investigations were repeated by DAS-ELISA (Clark and Adams, 1977). All available nepovirus isolates (ArMV, SLRSV, GFLV, GCMV, GBLV, AILV, GTRSV, GARSV, TRSV, ToRSV, TBRV) were assayed with the ELISA kit to N66. In addition, N66-infected grapevine and *C. quinoa* leaves were tested with eight commercial ELISA kits, three of which to ArMV (Agritest, Italy; Bioreba, Switzerland; Loewe, Germany) and one each to TBRV, ToRSV, SLRSV, RpRSV (all from Loewe), and with a locally produced kit to GFLV.

The ELISA kit to N66 was used for a preliminary field survey and for checking the possibile transmission of the virus through grapevine seeds.

Electron microscopy. Purified virus preparations were mounted in 2% aqueous uranyl acetate and observed within a Philips Morgagni electron microscope. Immunoelectron microscopy tests were done as described by Milne (1993). N66 particles were exposed to the homologous antiserum and to two antisera to ArMV whereas particles of seven ArMV isolates were exposed to N66 antiserum. For thin sectioning, tissue fragments excised from systemically infected leaves of *C. amaranticolor* were processed according to standard procedures (Martelli and Russo, 1984). Samples were fixed in 4% glutaraldehyde in 0.05M phosphate buffer pH 7.2, post-fixed at 4°C in 1% osmium tetroxide, dehydrated in graded ethanol dilutions and embedded in Spurr's resin. Thin sections were stained with lead citrate before observation.

Properties of purified virus. Coat protein (CP) subunits were dissociated from purified virus by boiling for 5 min in the presence of Laemmli's buffer (1.5% SDS, 20% glycerol and 3.5% 2-mercaptoethanol in 35 mM Tris-HCl buffer, pH 6.8). Protein samples were electrophoresed with a Protean II apparatus (Bio-Rad Laboratories, Hercules, CA, USA) in 12.5% and 5% polyacrylamide slab gels using a discontinuous buffer system and stained with Coomassie brilliant blue (Laemmli, 1970). Reference markers for molecular weight determi-

nation were standard protein subunits of MW-SDS 70L kit (Sigma-Aldrich, Milan, Italy), and coat protein subunits from other nepoviruses.

Nucleic acids were obtained from purified virus in Tris-EDTA buffer pH 7.5, by incubating at room temperature for 15 min in the presence of 1% SDS, then extracting twice with 1 vol. of TE-satured phenol and chloroform (Diener and Schneider, 1968). The aqueous phase was washed with 1 vol. of chloroform and nucleic acid was collected by ethanol precipitation overnight at –20°C. Electrophoresis was in 1.2% agarose gel in TBE buffer (22.5 mM Tris, 22.5 mM boric acid, 0.5 mM EDTA, pH 8.3) under semi denaturing conditions after denaturation with 50% formamide at 70°C for 3 min (Sambrook *et al*., 1989). Nucleic acid migration rate was measured with a standard Sigma RNA marker (2.9–9.6 Kb), in the presence of RNAs of the other nepoviruses used for comparison. Gels were stained with 1% ethidium bromide.

Cloning and sequencing of the 3' end of viral RNA-2. One mg of viral RNA was heat-denatured at 70°C for 3 min and primed by oligo(dT) to synthesize a blunt-end double stranded cDNA using a cDNA Synthesis Kit (Hoffmann-La Roche, Basel Switzerland) according to the manufacturer's instructions. The synthesized ds-cDNA was then ligated to a pUC18/*SmaI-Bap* plasmid (Amersham Biosciences, Freiburg, Germany). The resulting recombinant plasmid was cloned in *Escherichia coli* Top 10 competent cells and transformed cells were plated on Luria-Bertani medium containing 75 mg ml^{-1} ampicillin (Sambrook *et al*., 1989). Recombinant colonies were selected, plasmids purified (Holmes and Quigley, 1981), digested with *EcoRI* and *HindIII* restriction enzymes and analysed by agarose gel electrophoresis. DNAs of selected plasmids were automatically sequenced by MWG Biotech (Ebersberg, Germany). Nucleotide and protein sequences were analyzed with the assistance of the Strider 1.1 program (Marck, 1988). Sequences of the encoded proteins were aligned with Clustal W program (Thompson *et al*., 1994) and tentative phylogenetic trees were constructed with the NEIGHBOR, SEQBOOT, PROTDIST and CONSENSE programs of the PHYLIP package (Felsenstein, 1989).

Virus-specific PCR detection. Based on the sequenced portion of the CP cistron, virus-specific primers, namely N66-1: 5'-GGTCCTATAACATACGG GGCTG-3' (sense) and N66-2: 5'-CCCCTGATGGTGCTATTG-GTCC-3' (antisense), intended to amplify a genome fragment of 240 bp, were designed by computer analysis (Primer Selection Program, Henry M. Jackson Foundation, Bethesda), synthesized by MWG Biotech, and used for PCR assays from crude grapevine extracts (Wetzel *et al*., 1991). For comparative studies, crude extracts from grapevine plants infected with other nepoviruses were also used. For the synthesis of cDNA, 5 µl of the crude total nucleic acid extracts (TNAs) were primed with 1 µg of oligo (dT) after heat denaturation and reverse-transcribed with 200 units of Moloney murine leukemia virus reverse transcriptase (Invitrogen Corporation, Groningen, The Netherlands) in a 50 µl reaction for 1 h at 39°C. Five µl of synthesized cDNA were mixed with 45 µl of the amplification mixture [1x Taq Promega buffer, 1.5 mM $MgCl_2$, 0.2 µg of each primer 2.5, mM of each dNTPs and 1 unit of Taq DNA polymerase (Promega, Madison, WI, USA)]. An initial denaturation was done at 94°C for 2 min, and followed by 30 cycles as follows: denaturation for 40 sec at 94°C, annealing for 40 sec at 60°C, and extension for 45 sec at 72°C. A final extension was for 10 min at 72°C. PCR amplicons were analyzed by electrophoresis in 1.2% agarose in 1x TBE buffer and visualized by staining with ethidium bromide (Sambrook *et al.*, 1989).

Table 1. Reaction of herbaceous hosts to infection by isolate N66. Symbols: Ch = chlorotic; N = necrotic; D = deformation; Lt = latent; M = mottling; Vc = vein clearing; - = no infection.

Hosts	Local symptoms	Systemic symptoms
AMARANTHACEAE		
Gomphrena globosa	Lt	Lt
CHENOPODIACEAE		
Chenopodium quinoa	Ch	MVcD
Chenopodium amaranticolor	N	MVcD
Chenopodium bushianum	N	MVc
Chenopodium foetidum	Lt	Lt
SOLANACEAE		
Nicotiana benthamiana	Lt	Lt
Nicotiana cavicola	Lt	Lt
Nicotiana clevelandii	Lt	Lt
Nicotiana glutinosa	Lt	Lt
Nicotiana occidentalis	Ch	Vc
Datura stramonium	-	-
LABIATAE		
Ocymum basilicum	-	-

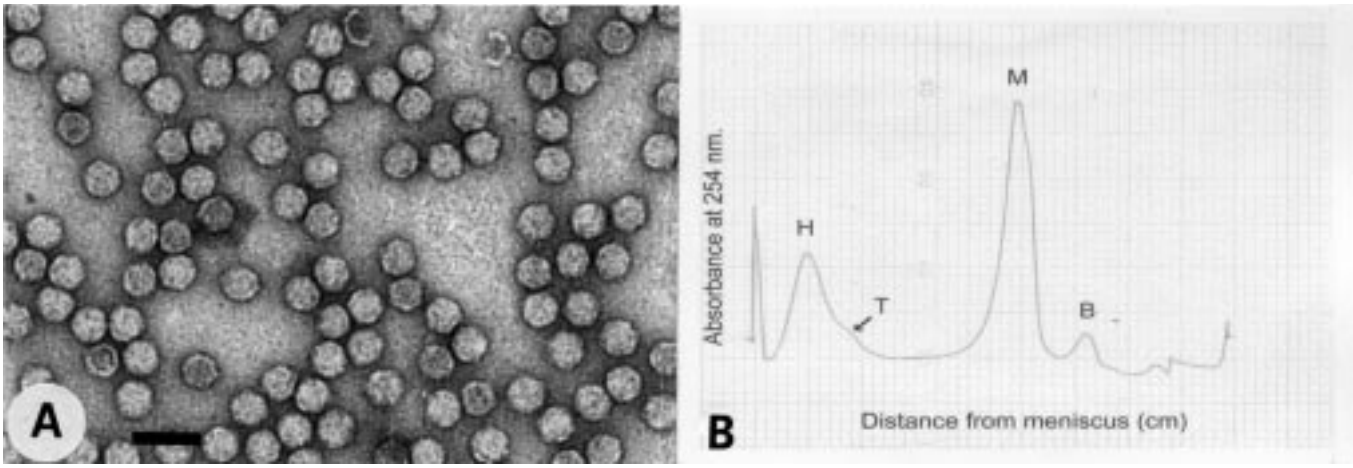

Fig. 2. A) Negatively stained N66 virus particles from a purified preparation. Bar = 50 nm. **B)** Sedimentation profile of N66 in sucrose density gradient centrifugation (sedimentation is from left to right). H = healthy plant components, T = top, M = middle, B = bottom.

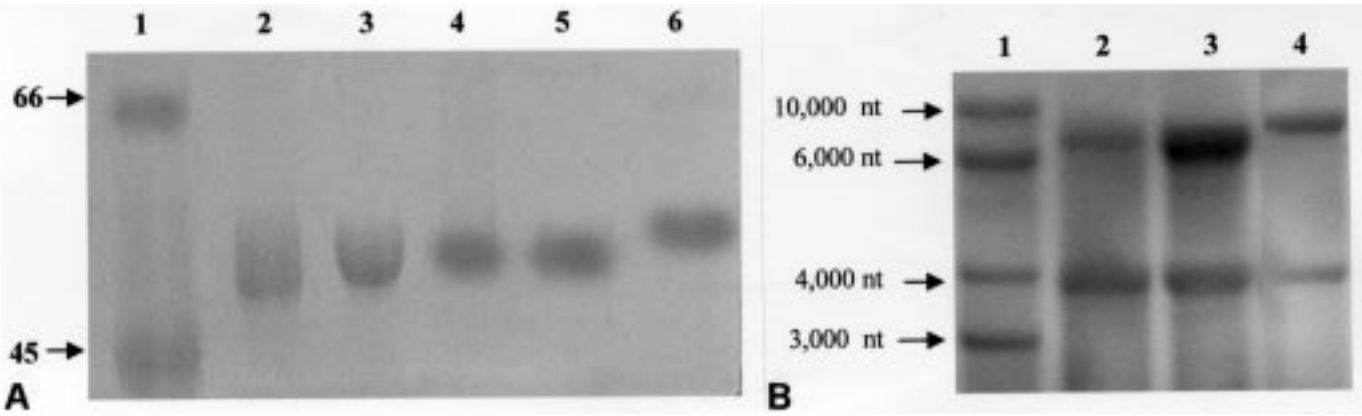

Fig. 3. A) Electropherogram of dissociated N66 coat protein (lanes 4 and 5) compared with coat proteins of other nepovirus species in subgroup A: GFLV (lane 2), ArMV (lane 3), RpRSV (lane 6). Reference markers in lane 1. **B)** Electropherogram of N66 genomic RNAs (lane 2) compared with RNAs of other nepovirus species in subgroup A: ArMV (lane 3), RpRSV (lane 4). Reference markers in lane 1.

RESULTS AND DISCUSSION

Host range and symptomathology. N66 infected 10 of 12 plant species in four different botanical families (Table 1). In all *Chenopodium* species, except for *C. foetidum*, which was infected symptomlessly, infection induced chlorotic or necrotic local lesions followed by systemic mottling and deformation of the leaves. The reaction of *C. amaranticolor* was strikingly similar to that elicited by ArMV and GFLV (Martelli, 1993). However, unlike these viruses, N66 did not induce symptoms in *Gomphrena globosa* and in most of the *Nicotiana* species assayed.

Virus purification. N66 was readily purified from *C. amaranticolor* tissues with average yields of *ca.* 10 mg/kg of tissue. Partially purified, unfractionated preparations contained two types of isometric particles *ca.* 30 nm in diameter, *i.e.*, empty shells penetrated by the stain and apparently intact particles with an angular contour and poorly resolved surface structure (Fig. 2A). Sucrose densitiy gradient centrifugation separated virus preparations into three components (Fig. 2B), empty shells (component T) and apparently intact virions (components M and B).

Analysis of virus proteins and nucleic acids. Electrophoretic migration rates and molecular weights of N66 dissociated CP subunits (*ca.* $54 \cdot 10^3$ Da) and RNAs (RNA-1, *ca.* $2.6 \cdot 10^6$ Da; RNA-2 *ca.* $1.3 \cdot 10^6$ Da) tallied with those of members of subgroup A in the genus *Nepovirus* (Wellink *et al.*, 2000), especially ArMV and GFLV (Fig. 3A and B).

Sequence analysis. The largest cDNA clone was 1,175 nt in size (GenBank accession number AY233975) and comprised the 3' terminal tract of RNA-2. This sequenced genome fragment represented an incomplete ORF encoding a polypeptide of 320 amino acids followed by a non-coding region. Direct comparison of this partially sequenced gene with available sequences from GeneBank revealed homologies with CPs of all sequenced members of the genus *Nepovirus*. In particular

N66 had 69% identity at the amino acid level with a comparable CP region of ArMV, 58% identity with GFLV, and 25% identity or less with other nepovirus species (*i.e.*, 18% with GARV and BLMV, 20% with AILV and TBRV, 22% with ApLRSV and TRSV, 23% with GCMV, and 25% with OLRSV)**.** Thus, the identity level of N66 CP amino acid sequence was always lower than 80%, which is the threshold for species demarcation in the genus *Nepovirus* (Wellink *et al.*, 2000). In a phylogenetic tree (Fig. 4) constructed with nepovirus CP sequences, N66 clustered with ArMV and other species of subgroup A (Wellink *et al.*, 2000).

Cytopathology. The structural organization of *C. amaranticolr* mesophyll cells was relatively well preserved (Fig. 5A). Chloroplasts and nuclei had a normal aspect, whereas mitochondria were damaged, showing a loose matrix and few or no cristae. Many cells contained cytopathological structures next to the nuclei, that resembled the vesiculate-vacuolate inclusions typically induced by nepovirus infections (Martelli and Russo, 1984) (Fig. 5A) and which are thought to be sites of virus replication (Pfeiffer *et al.*, 2000). N66-induced inclusion bodies consisted of compact accumulations of membranous vesicles with fibrillar content, endoplasmic reticulum strands, osmiophilic globular bodies with a smooth texture resembling lipid droplets, and virus particles (Fig. 5B). The appearance of these inclusions differed from that observed in cells infected by other nepoviruses (Martelli and Russo, 1984). Virions were either scattered or more often arranged in rows or in microcrystals in the cytoplasm (Fig. 5C) or close to and within plasmodesmata (Fig. 5D). Tubules containing particles, which are a common feature of nepovirus-infected cells, were not seen. Intracellular virus aggregates differed very much from those observed in GFLV-infected cells (Saric and Wrischer, 1975; Savino *et al.*, 1985) but had a slight resemblance to intracellular aggregates of ArMV (Gerola *et al.*, 1969; Savino *et al.*, 1985).

Serology. The antiserum to N66 had a titre of 1:1024, as determined by GTD, and gave a single precipitin line with the homologus antigen. It reacted with ArMV (Fig. 6A) to a dilution of 1:64 (serological differentiation index = 4) but not with any of the following nepoviruses GFLV, RpRSV SLRSV, GCMV, AILV, GBLV, GTRSV, GARSV, TRSV, TBRV, ToRSV (not shown). Similarly, no reaction was observed when partially purified N66 antigens were tested against the antisera to 17 different nepovirus species, which included all the above viruses plus AYRSV, BLMV, CLRV, ChYMV, MLRSV, and PRMV (not shown). In immunolectron microscopy tests, undiluted N66 antiserum decorated both homologous (Fig. 6B) and ArMV (Fig. 6C) particles. Whereas the homologous decoration was visible up to an antiserum dilution of 1:100,000, ArMV particles were decorated to a dilution of 1:1,000 (data not shown). With ELISA tests, the N66 antiserum detected the homologous antigen both in grapevine and herbaceous host extracts, but none of the above nepoviruses, except for ArMV isolate G-PFr22 in *C. quinoa,* which gave a very weak reaction (A_{405} absorbance value was twice that of healthy controls). Likewise, no reaction was observed in assays made with ELISA kits to TBRV, ToRSV, SLRSV, RpRSV, and GFLV. By contrast, a clear-cut response was obtained when N66-infected tissue extracts were exposed to each of three different commercial ELISA kits to ArMV (Agritest, Loewe, and Bioreba). ELISA was then extended to a larger number of grapevine ArMV grapevine isolates of different origin, including two Italian (I-IM299 and I-Ch), four French (F-En1, F-S2, F-N152 and F-6/29) and one German (G-PFr22) iso-

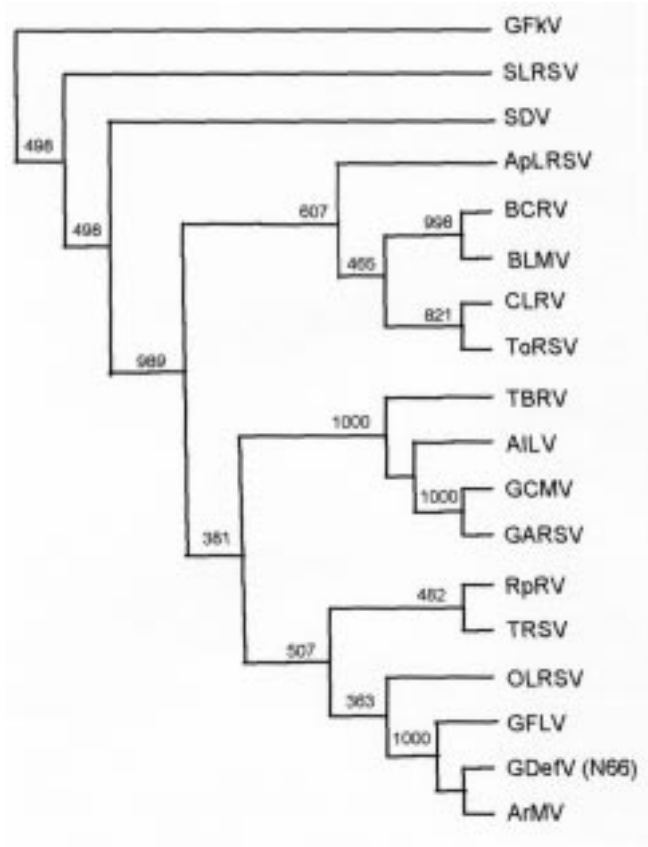

Fig. 4. Phylogenetic tree based on CP sequences of different nepoviruses, constructed and boostrapped with Clustal W programme. *Grapevine fleck virus* (GFkV, AJ309022) used as outgroup, Strawberry latent ringspot virus (SLRSV, X77466), *Satsuma dwarf virus* (SDV, AB009959), *Apricot latent ringspot virus* (ApLRSV, AJ278875), *Blackcurrant reversion virus* (BCRV, NC 003502), *Blueberry leaf mottle virus* (BLMV, U20621), *Cherry leaf roll virus* (CLRV, S63537), *Tomato ringspot virus* (ToRSV, D12477), *Tomato black ring virus* (TBRV, X80831), *Artichoke Italian latent virus* (AILV, X87254), *Grapevine chrome mosaic virus* (GCMV, X15163), Grapevine Anatolian ringspot virus (GARSV, AY218837), *Raspberry ringspot virus* (RpRV, AF226159), *Tobacco ringspot virus* (TRSV, L09205), *Olive latent ringspot virus* (OLRSV, AJ277435), *Grapevine fanleaf virus* (GFLV, AY017338), Grapevine deformation virus [(GdefV = N66) AY233975], *Arabis mosaic virus* (ArMV, AY017339).

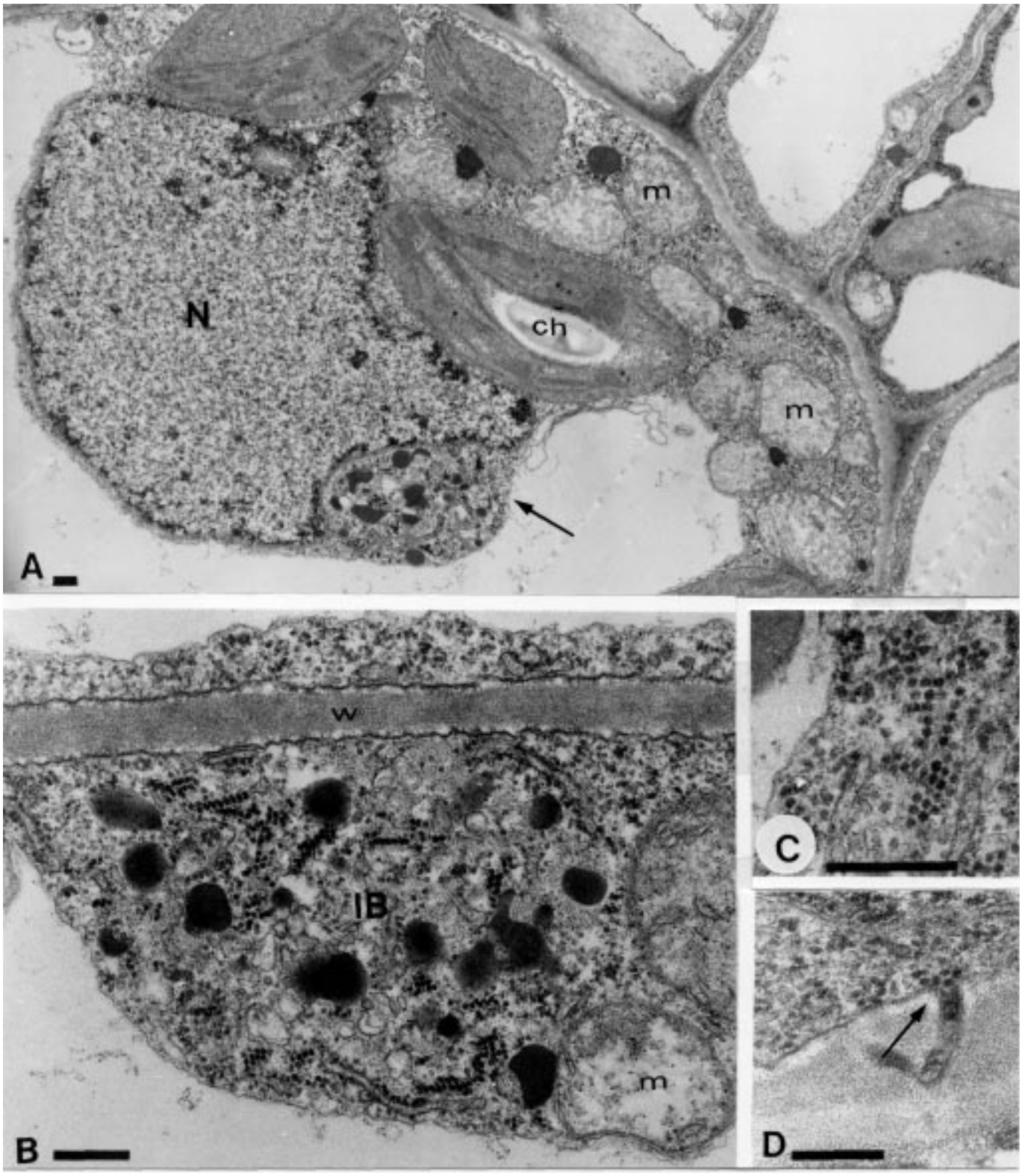

Fig. 5. Ultrastructure of N66 infections in *C. amaranticolor* tissues. **A)** Transection of a vascular parenchyma cell showing an apparently normal nucleus (N) with an inclusion body (arrow) apposed to it. The cytoplasm contains damaged mitochondria (m) and electron dense globules resembling lipid droplets, which were not seen in healthy controls. **B)** A close-up of an inclusion body (IB) made up of membranous vesicles, endoplasmic reticulum strands, osmiophilic globules and virus particles, which are scattered or arranged in rows or microcrystals. Ch =chloroplasts. **C)** Close-up of virus particle mycrocrystals. **D)** Virus particles next to and within the lumen of a plasmodesma (arrow). All bars = 250 nm.

Table 2. Results of a representative ELISA test made with infected *C. quinoa* and grapevine leaf extracts using the antiserum to N66 and a commercial ArMV kit (Agritest).

Accession	ELISA readings (A_{405}) with the anti-N66 serum [a]		ELISA readings (A_{405}) with the Agritest kit to ArMV [a]	
	Grapevine	*C. quinoa*	Grapevine	*C. quinoa*
ArMV isolates				
I-Ch	nt	0.258	nt	2.023
F-6/29	0.186	0.274	1.422	2.131
F-N 152	0.158	0.142	1.261	1.670
G-PFr22	0.175	0.287	1.965	2.180
F-En 1	0.164	0.255	0.816	2.030
F-S2	0.142	0.261	0.652	1.986
I-Im299	nt	0.195	nt	1.814
N66	1.908	2.164	0.541	1.052
Healthy control	0.140	0.146	0.140	0.145

[a] Average of four tests. Nt = not tested. ArMV isolates originated from Italy (I-IM299 and I-Ch), France (F-En1, F-S2, F-N152 and F-6/29) and Germany (G-PFr22).

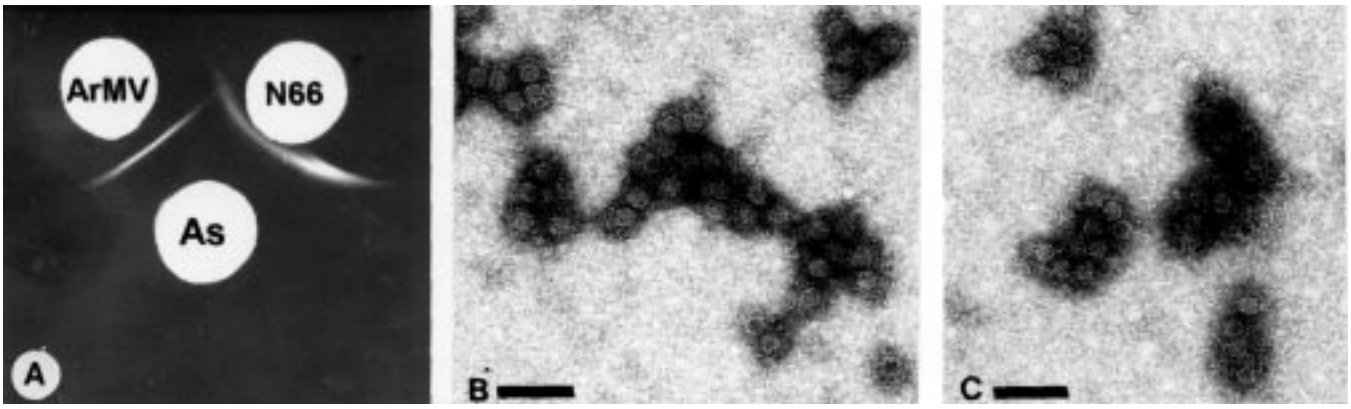

Fig. 6. Evidence of the serological relationship between N66 and ArMV. **A.** The undiluted antiserum to isolate N66 (As) cross reacts with both the homologous antigen and ArMV. N66 (A) and ArMV (C) particles decorated by undiluted antiserum to isolate N66. Bars = 100nm.

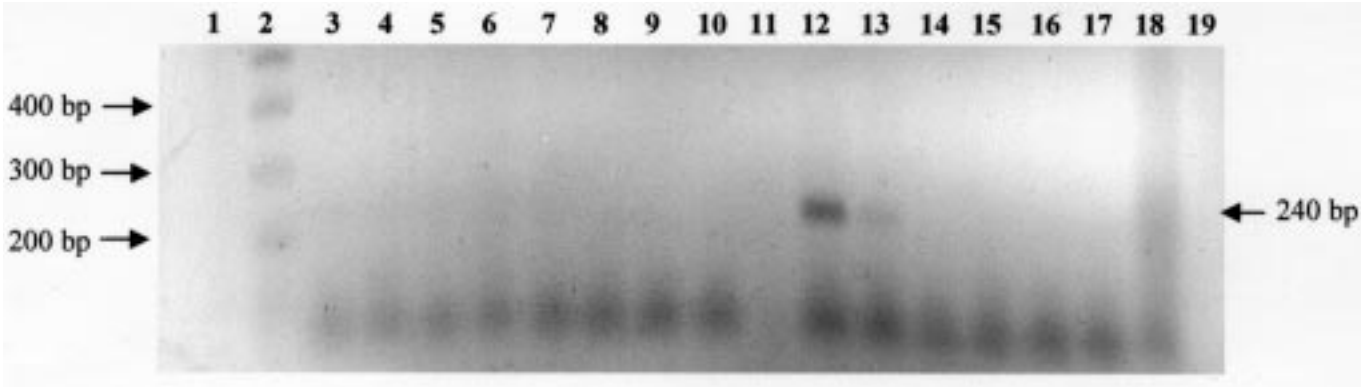

Fig. 7. RT-PCR detection of N66 in crude extracts from grapevine leaves, healthy (lane 1) or infected with: ArMV (isolate F-6/29 isolate) (lane 3), TBRV (lane 4), GFLV (lane 5). GCMV (lane 6), ToRSV (lane 7), GTRSV (lane 8), AILV (lane 9), RpRSV (lane 10), isolate N66 (lanes 12 and 13), SLRSV (lane 14), GBLV (lane 15), ArMV isolate G-PFr22 (lane 16), CLRV (lane 17), GARSV (lane 18). Water controls are in lanes 1 and 11 and reference markers in lane 2.

late. None of these isolates was detected in ELISA by N66 antibodies in grapevine leaf extracts, but very weak reactions (about twofold the healthy control) were given by some ArMV isolates (e.g. F6/29 and G-PFr22) in herbaceous host extracts (Table 2). By contrast, all ArMV isolates and N66 gave clear-cut positive responses to commercial ArMV ELISA kits (Table 2).

RT-PCR detection. In RT-PCR assays using grapevine crude leaf extracts, the primers designed on the available N66 sequence, amplified the expected 240 nt DNA fragment from samples collected from two different ELISA-positive vines. No amplification was obtained from negative controls (healthy grapevine) and grapevines infected with other nepoviruses, including ArMV (Fig. 7). The smear in lane 18 (GARSV) represents a non-specific reaction as no clear-cut band was ever obtained, and it did not appear in any of several other amplification experiments.

Field survey and seed transmission. In a preliminary survey carried out in vineyards of south-eastern Turkey from Adiyman to Salinurfa, N66 was detected by ELISA in 26 out of 757 vines (average infection 3.4%) of the following cultivars, Azezi, Besni, Kizlartahatasi, Sire, Siyah saraplik, Sirfoni, Köhnü, Ak Dimrit, Kara Dimrit, Cilores. N66 was not detected by ELISA in any of 187 seedlings from infected cv. Kara Dimrit seeds.

The results of biological, serological, physico-chemical, ultrastructural, and partial molecular characterization of isolate N66, strongly support the notion that this virus is a nepovirus serologically related with ArMV. However, N66 is sufficiently distinct from this and other members of the genus *Nepovirus* that infect grapevines, including those of subgroup A (GFLV, RpRSV, and TRSV), to warrant classification as an independent species, for which the name of Grapevine deformation virus (GDefV) is proposed. Like other nepoviruses infecting grapevine, in particular those of the European group (Walter and Martelli, 1997; Martelli, 1999), GDefV is associated with degeneration of vines both in the field and in the glasshouse.

ArMV has repeatedly been identified in Turkish grapevines by ELISA (Ozaslan *et al.*, 1995; Akbas and Erdiller, 1998; Cigsar and Yilmaz; 2000; Cigsar *et al.*, 2002), also in areas where GdefV is now known to occur. Since, as we have shown, commercial ELISA kits recognize GdefV in grapevine extracts, it seems plausible that some of these records refer to GDefV rather than to ArMV.

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