European Journal of Plant Pathology **107:** 677–684, 2001.

Incidence of viruses infecting *Allium* spp. in Greece

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Accepted 10 May 2001

Key words: Allexivirus, Garlic common latent virus, *Leek yellow stripe virus*, *Onion yellow dwarf virus*, *Shallot latent virus, Turnip mosaic virus, Allium* virus incidence

Abstract

A survey identified viruses infecting garlic, leek and onion crops and wild *Allium* species in Greece. Virus identification was based on ELISA, immunoelectron microscopy, and occasionally on RT-PCR. Samples of cultivated *Allium* species were collected from five districts, whereas samples of twenty-seven wild *Allium* species were also collected from all over Greece. *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV) were identified in 98.5% and 83.7% of all samples, respectively, and were found in all regions. Allexiviruses were also detected in all regions and their incidence ranged from 62.5% to 70.5% (depending on region and type of allexivirus). *Garlic common latent virus* (GCLV) was detected in samples from Arcadia (97.6%) and Evia (18.0%) and in one field in Larissa (23.0%). *Shallot latent virus* (SLV) was found only in two areas (Evros and Theva) and in fields planted with imported propagative material, from Iran and China. The incidence of virus-like symptoms in leek crops ranged from 10.0% to 90.0% in different regions and fields and all symptomatic plants were found to be infected by LYSV. *Onion yellow dwarf virus* was only found in seven symptomatic onion samples from southern Greece. *Allium ampeloprasum* spp. *ampeloprasum* and *Allium flavum*, were the only wild *Allium* species found to be infected with LYSV. Finally *Turnip mosaic virus* (TuMV) was found in *A. sphaerocephalon, A. guttatum, A. subhirsutum*, and *A. neapolitanum.*

Introduction

Due to its continuous vegetative propagation, garlic is usually infected by a large number of different viruses, which cause significant yield reduction. Two potyviruses, *Onion yellow dwarf virus* (OYDV) and *Leek yellow stripe virus* (LYSV), are the main symptom inducing viral pathogens of garlic crop and are reported to occur worldwide (Van Dijk, 1993a). LYSV and OYDV are transmitted by aphids in a non-persistent manner, and their spread is mainly attributed to non-colonizing *Aphid* species (Bos et al., 1978a). The effects of OYDV and LYSV infection on the yield of different garlic cultivars were studied and a reduction of bulb weight was reported ranging from 39% to 60%, and from 17% to 54% for each virus respectively (Lot et al., 1998). Furthermore, simultaneous infection with both viruses caused a further decrease of bulb weight and growth (Lot et al., 1998). *Turnip mosaic virus* (TuMV) has occasionally been reported to infect wild *Allium* species. Occurrence of TuMV has been reported in Yugoslavia infecting *Allium ampeloprasum* and *Allium roseum* (Stefanac and Plese, 1980), and in Israel infecting *A. ampeloprasum* (Gera et al., 1997).

Two carlaviruses infecting *Allium* species, *Garlic common latent virus* (GCLV) and *Shallot latent virus*

(SLV), have been identified in the past (Van Dijk, 1993b; Bos et al., 1978b). *Garlic latent virus* (GLV) isolated from garlic, first described in Japan (Lee et al., 1979), is now considered identical to SLV (Van Dijk, 1993b). There is also evidence that sequence variation of SLV depends on geographic location and not on the *Allium* host species (Tsuneyoshi et al., 1998a). GCLV has been reported to occur in most countries, but it is not endemic in Japan, Taiwan, and Thailand (Barg et al., 1994; Barg et al., 1997), while SLV was detected predominantly in Asia and Europe (Van Dijk, 1993b; Barg et al., 1994; Barg et al., 1997). These carlaviruses are also transmitted by aphids in a non-persistent manner (Van Dijk, 1993b; Barg et al., 1994; Barg et al., 1997), and have a wide host range within *Allium* species (Van Dijk, 1993b). Research in Germany indicated the occurrence of at least four serologically distinct types of SLV that either occur separately or in various mixtures (Barg et al., 1994). There is currently no available data concerning the effect of garlic carlaviruses on symptomatology, or yield loss of various garlic cultivars, however it is likely that co-infection with potyviruses, may induce further yield reduction as reported for SLV and LYSV in leek (Paludan, 1980).

Mite-borne viruses were first reported to infect *Allium* species by Razvjazkina (1971) and named *Onion mosaic virus* (OMV). *Onion mite-borne latent virus* (OMbLV) and *Shallot mite-borne latent virus* (SMbLV) (Van Dijk, 1991) were the first well described mite-borne viruses. Serological studies showed that they are related to *Shallot virus X* (ShVX) (Kanyuka et al., 1992; Van Dijk and Van der Vlugt, 1994). In addition, other mite-borne viruses such as *Garlic virus A* (GarV-A), *Garlic virus B* (GarV-B), *Garlic virus C* (GarV-C), and *Garlic virus D* (GarV-D) (Sumi et al., 1993) were included in this group because of their high sequence similarity to ShVX. Mite-borne viruses induce faint short stripes, mild mosaic or no symptoms in *Allium* spp. (Yamashita et al., 1996; Van Dijk, 1991; Van Dijk and Van der Vlugt, 1994) and are transmitted by the eriophyid mite *Aceria tulipae* (Van Dijk, 1991). These viruses were recently grouped in a new genus named *Allexivirus* belonging to the *sindbisvirus*-like virus supergroup together with potexviruses and carlaviruses (Hillman and Lawrence, 1995). Their genome organization and the predicted amino acid sequences of several open reading frames (ORFs) show similarities to Potex- and carlaviruses, but they are clearly distinct from these two genera (Sumi et al., 1993; Song et al., 1998). Included members of this genus are: ShVX (Kanyuka et al., 1992), GarV-A, GarV-B, GarV-C, GarV-D (Sumi et al., 1993) and *Garlic virus X* (GarV-X) (Song et al., 1998). Their economic significance to crop production has not been determined yet. In *Allium* spp., allexiviruses occur in complexes and this interferes with the production of species-specific antisera for virus identification. Research in Germany indicated the occurrence of at least five serologically distinguishable *Allexivirus* types (Barg et al., 1994).

Information on viral infection of *Allium* crops in Greece is limited (Salomon and Katis, 1996) and was based on a small number of field samples. Therefore, an extensive survey was carried out to study the occurrence of *Allium* infecting viruses in cultivated and wild *Allium* species. Virus identification was based on Enzyme-Linked Immunosorbent Assay (ELISA), on immuno electron microscopy (decoration), and on Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

Materials and methods

Sampling

A total of 1300 garlic plants were collected randomly from 39 fields at 12 locations, from the following five geographical districts of Greece: Arcadia (Peloponnese), Larissa and Evia (Central Greece), Thessaloniki (Macedonia) and Evros (Thrace). Each sample consisted of the tip of the youngest fully developed leaf. Samples of leek, onion and wild *Allium* species were collected as follows: (i) Leaves of 300 symptomatic leek plants, were collected from six different locations in Thessaloniki, Larissa and Evia. Leek plants were selected from each field. Three randomly distributed sectors of 1 m^2 size were selected and samples from ten plants showing virus-like symptoms were taken. All plants in each sector were counted and the proportion of symptomatic plants was determined. (ii) Nine symptomatic onion leaf samples were collected from Pirgos Ilea, (Peloponnese). Finally, (iii) 182 plants of 27 wild *Allium* species were collected from all over Greece in the years 1996–1998. These plants were maintained in the farm of the Agricultural Research Centre of Macedonia and Thrace, N.AG.RE.F, (Greek Gene Bank, 570 01 Thermi-Thessaloniki, Greece) and leaves were used for

testing. All leaf samples were tested by ELISA for virus infection within 1–3 days post-harvest.

ELISA detection

Antisera used in this study and related information are presented in Table 1. All *Allium* samples were tested by DAS- and TAS-ELISA, as described previously (Koch and Salomon, 1994; Barg, 1996). Samples were homogenized in PBS-Tween using an approximate ratio of 1 g tissue/10 ml buffer. IgGs of all antisera were purified by adsorption to protein-A (Sambrook et al., 1989). All antisera were diluted with PBS-Tween. Alkaline phosphatase–IgG conjugates were diluted with PBS-Tween containing 2% Polyvinyl Pyrrolidone (PVP) and 0.2% egg albumin. All incubation steps were performed at 37 °C, for 3 h, except for the incubation following sample addition, which was performed overnight at 4 °C.

Double antibody sandwich (DAS-) ELISA was routinely used for the detection of LYSV, GCLV, OYDV and allexiviruses using the following antisera: LYSV-R, AS 892, OYDV-214, GMbMV 920802, AS 1146, AS 1301, and anti-GarV-C. In all cases plates were coated by adding 100 µl of 1 µg/ml of IgGs in each well. Alkaline phosphatase-conjugated IgGs were diluted 1 : 1000, 1 : 500, 1 : 500 and 1 : 300, for conjugates against LYSV-R, allexiviruses, GCLV and OYDV-214 respectively. Triple antibody sandwich (TAS-) ELISA was carried out for routine detection of OYDV, SLV and TuMV. Plates were coated by adding (i) 100 µl of AS 935 (1 µg/ml) for OYDV detection, (ii) a mixture of AS 944 (3 µg/ml) and AS 991 (1 µg/ml) for SLV detection and, (iii) AS TuMV 7142 (1 µg/ml) for TuMV detection. Culture supernatants containing monoclonal antibodies were used for detection. (i) MAb 5G1 (diluted 1/100) for OYDV detection, (ii) MAbs 5B5, 2G3, and 6A9 (each diluted 1/100), were combined for SLV detection, and (iii) MAb EMA 67 (diluted 1/2500) for TuMV detection. Alkaline phosphatase-conjugated goat anti-mouse IgGs were diluted 1 : 2500. All other antisera included in Table 2 were evaluated for their sensitivity and occasionally used for verification purposes, using an antigen coated plate (ACP-) ELISA protocol (Koch and Salomon, 1994). Virus free garlic cloves, used as healthy controls, were a kind gift from Dr. K. Masuda (Hokkaido University, Sapporo, Japan), and from N. Leventakis (VITRO HELLAS Company, 593 00 Nisseli Imathias, Greece).

Table 1. Specific antisera used for detection of *Allium* viruses and their origin

Specificity	Antisera/ MAbs	Original *Allium* host	Produced by
LYSV	AS 1083	leek	Barg E.
LYSV	AS LYSV 970221	garlic	Yamashita K.
LYSV	AS LYSV-R	leek	Lot H.
LYSV	AS Anti-GPV-7*	garlic	Sumi S.
OYDV	AS 935	onion	Barg E.
OYDV	MAb 5G1	onion	Barg E.
OYDV	AS OYDV-214*	garlic	Salomon R.
GCLV	AS 892	garlic	Barg E.
SLV	AS 944	*A. fistulosum*	Barg E.
SLV	AS 991	leek	Barg E.
SLV	MAb 5B5	shallot	Barg E.
SLV	MAb 2G3	shallot	Barg E.
SLV	MAb 6A9	seek	Barg E.
Allexivirus spp.	AS 1146	garlic	Barg E.
GarV-D	AS 1301	garlic	Barg E.
Allexivirus spp.	AS GMbMV 920802	garlic	Yamashita K.
GarV-C	AS Anti-GarV-C*	garlic	Sumi S.
TuMV	MAb EMA 67	—	Jenner C.
TuMV	AS TuMV 7142	—	(IPO-DLO)

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Dr. Herve Lot, *INRA* (Monfavet, France).
Dr. Shin-Ichiro Sumi, Institute for biotechnology Research, Wakunaga Pharmaceutical Co. Ltd., Hiroshima, Japan.
Dr. Carol Jenner, Horticulture Research International, Wellsbourne–Warwick, UK.
*Indicates antisera raised against bacterially expressed CPs.

Immunoelectron microscopy

Identification of viruses was done by trapping particles from crude leaf extracts in 0.1 M phosphate buffer, pH 7.0, on copper grids for 15 min, followed by a 15-min-incubation with antiserum, diluted 1/50, for decoration. Trapped virions were stained with 1% (w/v) uranyl acetate solution.

RT-PCR

RT-PCR for OYDV detection in nine onion samples was performed using plant crude extracts. Extracts were prepared by grinding 0.1 g of tissue in 1 ml extraction buffer (50 mM Tris–HCl, [pH = 8.3],

Table 2. Incidence of viruses in garlic crops in different regions of Greece, as determined by different antisera in DAS- and TAS-ELISA

Province	Number of samples	MAb 5G1/ AS 935	AS LYSV-R	AS 892	MAbs 5B5, 2G3, 6A9/ AS 944, AS 991	AS 1301	AS anti-GarV-C	AS 1146	AS GMbMV 920802	MAb EMA 67/ AS TuMV 7142
		OYDV (%)	LYSV (%)	GCLV (%)	SLV (%)	GarV-D (%)	GarV-C (%)	*Allexivirus* (%)	*Allexivirus* (%)	TuMV (%)
Evros	239	95.9	72.7	0	2.7	38.8	35	35	40.6	0
Thessaloniki	213	97.7	34.5	0.58	0	60.6	63.4	63.4	75.6	0
Larissa	415	99.5	93.5	3.1	0	98	89.4	89.4	97.6	—
Evia	222	100	98.6	18	0	92.3	78	78	82	—
Arcadia	211	99.5	100	97.6	0	15.5	19.3	19.3	29.4	—
Total	1300	98.5	83.7	20.7	0.5	67.3	62.5	62.5	70.5	0

75 mM KCl, 2% PVP), followed by centrifugation at 10 000 × *g* for 2 min. Complementary DNA (cDNA) was prepared in a 15-µl-reaction volume, using 1 µl of plant extract. Maloney Murine Leukaemia Virus Reverse Transcriptase (M-MLV RT, Life Technologies™, MD, USA), and oligo-$(dT)_{18}$ primer, were used according to the suppliers' instructions along with 30 units RNASEOUT (Life Technologies™). Twenty µl PCR reactions were performed using *Taq* DNA Polymerase (Life Technologies™), according to the manufacturer's recommendations and OYDV specific primers, OYDV 1 (5′-GAA GCA CAY ATG CAA ATG AAG G-3′) and OYDV 2 (5′-GCC ACA ACT AGT GGT ACA CCA C-3′) at a final concentration of 0.2 µM each. The cycling profile consisted of a first denaturation step at 94 °C, for 5 min; 35 cycles segmented in 30 s at 94 °C; 30 s at 58°C, and 30 s at 72°C; and one final extension step at 72 °C, for 10 min.

Results

Incidence of viruses in Greece, as determined by ELISA and PCR tests

All garlic crops surveyed were showing virus-like symptoms. Most plants were infected with a mixture of up to five different viruses. Based on ELISA tests, the incidence of LYSV and OYDV in garlic was 83.7% and 98.5%, respectively. These potyviruses were the most abundant garlic viruses in all examined regions. SLV was detected only in fields in Theva (ten positive samples, data not shown) and Evros, which were cultivated with propagative material imported from Iran and China, respectively. All samples from these fields were infected with SLV. GCLV was found only in Arcadia and Evia in 97.6% and 18.0% of the samples, respectively, while in Larissa GCLV was detected only on a field cultivated with propagative material imported from Italy and in Thessaloniki in one field in very low incidence. It seems that the occurrence of GCLV is restricted to southern and central Greece. The incidence of allexiviruses ranged from 15.5% to 98.0% depending on *Allexivirus* species and the region. AS 1301 specifically detects GarV-D (Barg, Vetten, and Lesemann, unpublished). The incidence of GarV-C was on a similar level as the incidence of GarV-D (Table 2). AS 1146 and AS anti-GarV-C gave identical detection results. All samples positive with both antisera were also positive with AS GMbMV 920802, but the GMbMV antiserum reacted positively with an additional number of samples. In Larissa and Evia, *Allexivirus* infection rates were close to 100%, whereas in Thessaloniki, Evros and Arcadia, *Allexivirus* infection was lower (Table 2). TuMV was not detected in garlic.

In leek no multiple infection was detected (as was possible by the range of antisera used), but all samples were infected by LYSV only. According to visual assessments in the field, the incidence ranged from 10% to 90% (Table 3) and it was higher in fields located near overwintering leek crops.

More than 30 onion fields were inspected in Greece, but in only one case plants were found exhibiting virus-like symptoms. This was a single field in the Peloponnese, where plants showed severe stunting, curling and leaf yellowing. Application of RT-PCR revealed the presence of OYDV (Figure 1). MAb 5G1, and antiserum OYDV-214 failed to detect OYDV infection in these onion samples.

Table 3. Incidence of LYSV in symptomatic leek samples collected from different areas in Greece as determined by ELISA

Province	Incidence (%) visually assessed	Virus	Number of samples tested	Number of positive samples
Thessaloniki	10–90*	LYSV	240	240
Larissa	20	LYSV	40	40
Evia	20	LYSV	20	20

*Range of incidence on different fields inspected.

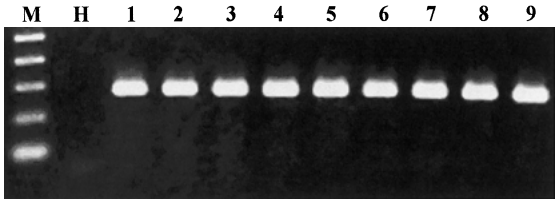

Figure 1. Gel-electrophoretic analysis of PCR products obtained from onion samples (1–9) infected by OYDV, using leaf extracts in a double tube RT-PCR. (H: healthy control, M: 100 bp DNA ladder). It should be noted that the obtained amplicons have the expected size (ca. 282 bp).

Of all wild *Allium* plants tested only *A. ampeloprasum ssp. ampeloprasum* and *A. flavum*, were infected with LYSV (Table 4). Both plants showed severe yellow striping. TuMV was found in *A. sphaerocephalon* (showing mild yellow striping), and in *A. guttatum, A. subhirsutum, and A. neapolitanum* (all three plants showed severe yellow striping).

Decoration tests

Samples tested by EM were usually simultaneously infected with different viruses. This was evident from their distinct particle morphology and specific decorations. Filamentous particles, more flexuous than those of the two potyviruses, were observed in the case of *Allexivirus* infection. Filamentous virions, usually straight or slightly curved were observed in the case of *Carlavirus* infection such as SLV and GCLV. Filamentous particles were decorated using Mab EMA 67, in the case of TuMV infection in wild *Allium* plants.

Discussion

Garlic is an economically important crop for several Greek agricultural regions. Greek growers traditionally produce their own garlic propagative material. This fact accounts for the observed heavy viral infection and implies a potentially high reduction in yield and quality of this crop. Additionally, a high incidence of virus-like symptoms in leek crops was observed in Greece. To face this problem, a strategy is currently under development in Greece, for the production of virus-free garlic propagative material. An epidemiological survey has been carried out in order to identify *Allium* infecting viruses and investigate their distribution in Greece, because only limited data are currently available. Consequently, this study aimed at the identification of *Allium* infecting viruses and determining their distribution in Greece. Previous reports from different countries were published presenting surveys of a smaller number of viruses and areas. This is the first report of an extended survey including most of known viral pathogens of *Allium* crops.

Table 4. Virus incidence (OYDV, LYSV, GCLV, SLV, and allexiviruses) in wild Allium species

Wild *Allium* species	Virus/no. of plants	No. of samples
A. vineale	—	17
A. guttatum	TuMV/1	41
A. ampeloprasum	—	27
A. ampeloprasum spp. *ampeloprasum*	LYSV/1	2
A. flavum	LYSV/1	11
A. sphaerocephalon	TuMV/1	20
A. sphaerocephalon spp. *aegaeum*	—	1
A. amethystinum	—	1
A. subhirsutum	TuMV/1	1
A. neapolitanum	TuMV/1	1
A. lagarophyllum	—	1
A. pallens	—	5
A. chamaespathum	—	2
A. achaium	—	1
A. paniculatum	—	4
A. tardans	—	1
A. euboides	—	3
A. commutatum	—	2
A. statisiforme	—	4
A. automnale	—	1
A. longanum	—	1
A. integerrinum	—	2
A. trifoliatum	—	1
A. scorodoprasum	—	6
A. dentiferum	—	3
A. cepa	—	1
A. spp.	—	22
Total	LYSV/2, TuMV/4	182

Our results show that garlic in Greece is heavily infected with viruses. OYDV and LYSV, probably the most important viruses of garlic, were found to be also the most abundant and widespread viruses in Greece (up to 100%). Significant differences were found regarding the frequency and the distribution of the other garlic viruses. GCLV is restricted to southern Greece and SLV was found at a low percentage only in areas, where propagative material was imported from China and Iran. Therefore, SLV does not seem to be originally endemic in Greece. Allexiviruses are found only in garlic and their regional distribution was irregular. In three out of five regions, their incidence is very high (up to 100%). The spread of allexiviruses seems to take place primarily during bulb storage, where mites can easily spread, and scarcely in the field (Lange and Mann, 1960). If the farmers use their own material as it happens in Greece, 100% infection is most likely to occur. In Greece, incidence of carlaviruses in garlic is lower than that of potyviruses. This can be explained by the observation of Van Dijk (1993b), that aphids transmit carlaviruses less effectively than potyviruses. It is also possible, that carlaviruses are mainly transmitted by vegetative propagation. The planting material used in Arcadia is genetically different from that used in the other areas and high incidence of GCLV might be attributed to its high susceptibility or to a high transmission efficiency of the virus strains by aphids.

All-year-around cultivation of leek is a common practice in Greece, and it seems to be the main reason for the high incidence of LYSV in leek. No other virus was found infecting leek. It should be stressed though, that only symptomatic leek samples were collected, and since allexi- and carlaviruses cause latent infections in alliums, sampling was biased for detecting primarily LYSV infections.

Virus incidence in onion seems to be low, since only seven onion plants were found, exhibiting virus-like symptoms. The plants were infected solely with OYDV, probably with the onion isolate (van Dijk, 1993a). OYDV in onion is no longer of economic importance in Western Europe. This is due to control measures, especially the introduction of a period free of onion crop, to prevent an overlapping cultivation of diseased crops and new plantings (Walkey, 1990). The use of onion plants raised from seeds also plays an important role for the reduction of virus infections of onions, because *Allium* viruses are not seed-transmitted. The low infection rate of onions in Greece is probably due to the seasonal isolation of this crop. Usually the crop is harvested from June until the end of August and the establishment of new crops begins at the earliest in October.

Since allexi- and carlaviruses have a wide host range within the alliums, including wild species, these could serve as a source of viral inoculum for the cultivated ones. Therefore, the survey was extended to determine virus presence in wild alliums. In a previous report, *Allium* spp. in the Netherlands were not infected with typical *Allium* viruses, and do not appear to be a source of viral infection in *Allium* crops (Van Dijk, 1991; 1993a,b). In this study, a similar number of wild alliums were tested, which comprised a larger number of species. Allexi- and carlaviruses were not detected in wild alliums. TuMV and LYSV were the only viruses detected (Table 4). Since TuMV was not detected in garlic or leek crops in Greece, it is probably restricted to some wild *Allium* spp. We have not tested whether LYSV isolates from wild *Allium* species can infect cultivated *Allium* crops.

None of the antisera used in this study detected all allexivirus isolates, indicating the existence of distinct serotypes of these viruses (See also Barg et al., 1994). Concerning the specificity of antisera used against allexiviruses, results indicate that AS 1146 probably detects GarV-C strains, while GMbMV 920802 detects GarV-C and additional *Allexivirus* species, such as GarV-B. This was discovered during the examination of a sample reacting positively with GMbMV 920802 antiserum only. Cloning and sequencing of a 1000 bp fragment from this isolate revealed a 90% identity to corresponding fragment of GarV-B and 71% to the one of GarV-C (data not shown). Most probably the GMbMV antiserum was raised against a mixture of serologically distinct allexiviruses, since the purified virus preparation originated possibly from a garlic plant with a mixed infection. GMbMV was isolated from garlic via serial local lesion transfer on *C. murale* and back inoculated on virus free garlic. Either the isolation was not successful or the garlic was still infected with allexiviruses other than GMbMV, which, based on sequence comparisons, is a strain of GarV-C. However, regarding the specificity of an antiserum, the ultimate proof could be achieved only by conducting decoration tests with pure isolates. European antisera against LYSV originating from France and Germany (Barg et al., 1994; Barg, 1996), gave higher readings in ELISA with Greek samples, than antisera originating from Japan (Yamashita et al., 1996;

Tsuneyoshi and Sumi, 1996). Sequence comparisons of *Allium* potyviruses revealed a significant variability of OYDV and LYSV coat protein sequences (Tsuneyoshi et al., 1998b), and especially of the highly variable N′, which is the main antigenic determinant of potyviruses. This might result in lower affinity of the antibody produced against non-homologous virus isolates and may be the cause of higher OD readings of the Greek isolates with the antisera produced against European isolates. OYDV-214, a polyclonal antiserum produced against bacterially expressed coat protein (CP) (Salomon, unpublished results), reacted highly specific but gave low OD readings when it was tested with Greek isolates in DAS-ELISA. This may be explained by the above mentioned amino acid sequence variation, or alternatively by a lack of proper folding of the bacterially expressed CP. In an attempt to face the serology-related problems a RT-PCR assay was successfully employed for OYDV detection in onion.

A strategy to use virus-free garlic in field production is under way in Greece. Information concerning the incidence of garlic viruses is very important for routine virus indexing of virus-free propagative material, indicating the need for extensive testing for the presence of OYDV, LYSV and *Allexivirus* species rather than SLV and GCLV.

Acknowledgements

C.I. Dovas thanks the Greek scholarship foundation for economic support. This work was partially financed by a research project for the development of industrial research (PAVE) No. 321 of the General Secretariat of Research and Technology (GSRT). The authors thank Prof. P.E. Kyriakopoulou (Agricultural University of Athens, Plant Pathology Lab.) and S. Samaras (N.AG.RE.F., Agricultural Research Centre of Macedonia and Thraki, Greek Gene Bank, 570 01 Thermi-Thessaloniki, Greece), for providing virus infected onion and wild *Allium* samples respectively, and Dr. K. Masuda (Hokkaido University, Sapporo, Japan), and Mr. N. Leventakis (VITRO HELLAS, Company 593 00 Nisseli Imathias Greece) for roviding virus-free garlic cloves. Thanks are also due to Dr. A Frank (ARO Volcani Center) for performing the immunoelectron microscopy and to Dr. K. Yamashita, Dr. Jan Vink, Dr. Masanobu Fukami, Dr Lot Herve, and Dr. Shin-Ichiro Sumi for providing antisera.

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