

Molecular detection and genetic diversity of potyviruses in *Allium* spp. in the Marmara Region of Türkiye and determination of onion and garlic genotypes' reactions to Onion yellow dwarf virus

Fatma ŞAFAK

fatma.safak@tarimorman.gov.tr

Atatürk Horticultural Central Research Institute: Atatürk Bahçe Kulturleri Merkez Arastirma Enstitusu Mudurlugu <https://orcid.org/0000-0002-4671-3730>

Gassan KÖKLÜ

Tekirdag Namik Kemal Universitesi Ziraat Fakultesi

Research Article

Keywords: Potyvirus, OYDV, LYSV, *Allium*, viruses

Posted Date: June 19th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4501239/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Garlic (*Allium sativum* L.), onion (*Allium cepa* L.), and leek (*Allium porrum* L.) are widely distributed in the Marmara region of Türkiye. *Leek yellow stripe virus* (LYSV) and *Onion yellow dwarf virus* (OYDV) are two of the most significant viral pathogens impacting the cultivation of edible *Allium* plants worldwide, leading to substantial economic losses. To assess the prevalence of these viruses, leaves from onion, garlic, and leek plants were collected from 778 plants across production fields in the Marmara region (Bursa, Edirne, Kocaeli, Kırklareli, Sakarya, and Tekirdağ). Samples were tested for the presence of OYDV and LYSV by ELISA and RT-PCR. Of the samples analyzed, 175 were found positive for OYDV, 79 for LYSV, and 21 showed mixed infection with both viruses. Successful amplification of specific gene regions (Nlb-CP for *Onion yellow dwarf virus* and CP for *Leek yellow stripe virus*) was achieved using specific primers. Sequence analysis revealed that the LYSV and OYDV isolates obtained in this study exhibited identities ranging from 84.6–98.1% and 74.1–99.8% with sequences available in GenBank, respectively. Additionally, the reactions of the genotypes to OYDV were also investigated. OYDV isolate TR-OK64 (OR923732) from Bursa was inoculated into 6 garlic and 15 onion genotypes sourced from the Atatürk Horticultural Central Research Institute. Statistical analyses conducted using JMP Pro 13 revealed infected plants' reductions in leaf length, bulb height, bulb width and bulb mass.

Introduction

Allium species are important vegetables that have been cultivated for thousands of years for use of their taste, aroma, and additionally to treat diseases due to their bactericidal, anticarcinogenic, and hypoglycemic properties (Galmarini 2018, Oommen et al. 2004). Viruses are significant causal agents of plant diseases, leading to reduced yields in *Allium* species. Among the most common viruses affecting the Alliaceae family are *Potyvirus*es, *Carlavirus*es, and *Allexivirus*es. They share a single-stranded RNA structure, yet their genome sequences differ. (Chen and Adams 2001). Although viruses that infect *Allium* plants may not kill them, they can significantly reduce yields over time, sometimes by more than 50% (Lot et al. 1998; Conci et al. 2003). *Leek yellow stripe virus* (LYSV) and *Onion yellow dwarf virus* (OYDV) are significant potyviruses that infect *Allium* species, leading to considerable economic losses (Shahraeen et al. 2008; Cremer et al. 2021). *Onion yellow dwarf virus* (OYDV) is commonly found in onion and garlic. The most prominent symptom of *Onion Yellow Dwarf Virus* (OYDV) is onion stunting, which leads to significant reductions in leaf width, leaf length, stem height, and seed diameter. In garlic, young foliage may exhibit irregular yellow streaking, mosaic-like patterns, as well as symptoms of dwarfing, and diminished seed quality and flower production. (Bos 1976; Entezari et al. 2021). OYDV transmitted by over 50 species of aphid species, vegetative production material, and mechanical inoculation. LYSV is a flexible and filamentous virus, approximately 815–820 nm long, a member of *Potyvirus* genus in *Potyviridae* family. OYDV natural hosts include leek, ornamental plants, garlic, onion, and wild *Allium* species (Diekmann 1997). LYSV-like symptoms were first reported in Germany in 1937 and later in the Netherlands in 1970 and later in the following years the symptoms reported from other European countries (Bos et al. 1978). LYSV causes mosaic symptoms, yellowing, and stripes on plants (Vucurovic et al. 2017). LYSV infected garlic plants exhibits stunting symptoms of the plants and light yellow stripes on leaves (Gupta et al. 2017). There are differences in the symptoms caused by OYDV in onions due to the possible presence of different variants in the virus population, as well as the impurity of commercially grown local onion varieties (Abubaker et al. 2019). OYDV was reported in onion growing areas from Iran (Shahraeen et al. 2008), Italy (Mangli et al. 2014), Serbia (Bagi et al. 2012), Indonesia (Harti et al. 2020), Ethiopia (Kebede et al. 2020), France (Lot et al. 1998), Ukraine (Sherevera et al. 2019), Czech Republic (Klukackova et al. 2007), Australia (Cremer et al. 2021), Pakistan (Gilani et al. 2016), Northern Cyprus (Yılmaz and Fidan 2021), Argentina (Conci et al. 2003), China (Chen and Adams 2001), Tunisia (Ayed et al. 2019), Croatia (Voncina et al. 2016) and Spain (Fernandez-Tabanera et al. 2018). LYSV was determined in research studies carried out in *Allium* species in Iran (Shahraeen et al. 2008), India (Gupta et al. 2017), Serbia (Vucurovic et al. 2017), LYSV in France (Lot et al. 1998), Ukraine (Sherevera et al. 2019), Czech Republic (Klukackova et al. 2007), Australia (Cremer et al. 2021), Pakistan (Gilani et al. 2016), Northern Cyprus (Yılmaz and Fidan 2021), Poland (Winiarczyk et al. 2014), China (Chen and Adams 2001), Tunisia (Ayed et al. 2019) and Croatia (Voncina et al. 2016). Enzyme-linked immunosorbent assay (ELISA) is a commonly used method for detecting viruses in *Allium* species. However, RT-PCR is a more sensitive diagnostic method, being 10–100 times more sensitive than ELISA (Arya et al. 2006). This study aimed to identify OYDV and LYSV using serological and molecular methods in onion, garlic, and leek plant samples in some production areas of the Marmara Region, Türkiye. At the same time, the phylogenetic relationships with the isolates registered in the NCBI GenBank and the reactions of the genotypes against the virus were determined as a result of inoculation of OYDV to onion and garlic genotypes.

Material and Methods

Collection of samples

Samples were collected from onion (*Allium cepa* L.), garlic (*Allium cativum* L.) and leek (*Allium porrum* L.) growing areas in Bursa, Tekirdag, Kırklareli, Sakarya, Edirne, and Kocaeli provinces in the Marmara Region (Fig. 1). The plant samples exhibiting viral symptoms such as growth retardation, small and misshapen bulb formation, general yellowing of the leaves, yellow or irregular chlorotic lines, ring-shaped discolouration, irregular lines parallel to the mid-vein, stunting, deformities, and narrowing of the stem were collected from onion and garlic fields in April, May, and June, and from leek fields in July, August, and September between 2020 and 2021 years.

Serological studies

DAS-ELISA was used to test onion, garlic, and leek leaves for OYDV and LYSV using antisera obtained from Bioreba GMBH, and carried out as described by in the Company's protocol. Absorbance values were measured at 405 nm wavelength using an ELX800 Universal Microplate Reader. Samples with absorbance values 2 times or more higher than the negative control value were considered infected.

Molecular Studies

RNA isolation and cDNA synthesis

RNA isolation was carried out using the Thermo Scientific Fisher Genejet Plant RNA Purification (USA) kit following the recommended protocol. Total RNA was extracted from 100 mg of leaf tissue of each sample was weighed and placed in the tube with the balls. The plant tissues were pulverised after cooling in liquid nitrogen for 1–2 minutes. The RNA was isolated directly from freshly prepared. The resulting RNAs were utilized for cDNA synthesis. For each sample, 20 µl volume was prepared containing 1 µl Random Hexamer Primer, 6 µl nuclease-free water, 5 µl total RNA, 1 µl RiboLock RNase inhibitor, 1 µl RevertAid M-Mulv Reverse Transcriptase, 2 µl dNTP Mix and 4 µl reaction buffer, following the Thermo Scientific RevertAid cDNA synthesis kit protocol.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) were carried out using isolates found positive results in DAS-ELISA. RT-PCR were conducted using oligonucleotides specific to the CP (coat protein) of *Leek yellow stripe virus* and the Nib-CP gene regions of *Onion yellow dwarf virus*. Primers used in this study were OYDV Nib-(F) CAC CNT AYA TAG CRG ARA CAG CT and OYDVCP-(R) CTA CTG AAA TGC GCC ATT ATY TGY CTA (Nam et al. 2015) amplify approximately 602 bp and LYSV-(F) CGC ATA TGC AGG GAT GTT TCG GTT and LYSV-(R) ATC AGC ATT CAG GCT GCT TAT ACA C (Nam et al. 2015) amplify 317 bp. Thermo Scientific Verso 1-Step RT-PCR kit were used for OYDV and Ampliqon brand Taq OptiMix CLEAR 2x Master Mix 1.5 mM MgCl₂ final concentration RT-PCR kit used for LYSV. The reactions were performed in two steps following the manufacturers' recommendations. For One-Step RT-PCR analysis used to diagnose OYDV, a 25 µl volume was prepared containing 0.5 µl Verso Enzyme Mix, 12.5 µl 2X 1-Step PCR ReddyMix, 1.25 µl RT Enhancer, 1 µl Primer F, 1 µl Primer R, 6.75 µl nuclease-free water, and 2 µl RNA. The RT-PCR used to detect the OYDV included one cycle of 15 minutes for cDNA synthesis at 50°C, followed by one cycle of 2 minutes at 95°C, 35 cycles of 20 seconds at 95°C, 30 seconds at 54°C, and 1 minute at 72°C, and finally 1 cycle at 72°C for 5 minutes. For LYSV detection, a 50 µl mix was prepared for the RT-PCR reaction, consisting of 25 µl Taq 2x Master Mix, 1 µl Forward Primer (10 µM), 1 µl Reverse Primer (10 µM), 21 µl Nuclease-free H₂O, and 2 µl Template DNA (cDNA). The RT-PCR conditions for LYSV involved one cycle of 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, and finally one cycle of 72°C for 5 minutes. PCR product was separated in a 1.5% agarose gel and stained in SafeView Classic. Electrophoresis was performed at 100 V for 60 minutes to run the samples.

Sequence analysis

Molgentek (Adana, Türkiye) conducted sequencing studies to determine the partial nucleotide sequences of selected virus isolates, following RT-PCR. PCR products of virus isolates underwent pairwise sequencing (Table 1). Sequence variability within the obtained OYDV and LYSV isolates were investigated and Mega.6 (Tamura et al. 2013) was used to compare the isolated sequences to the sequences obtained from GenBank.

Table 1
List of OYDV and LYSV isolates used for sequencing

Sample Name	Primer Name	Obtained product length (bp)	Product Name	City/District
TR-p17	LYSV	317	Leek	Bursa/Karacabey
TR-24	LYSV	317	Garlic	Kırklareli/Vize
TR-p55	LYSV	317	Leek	Bursa/Karacabey
TR-m82	LYSV	317	Garlic	Edirne/Kesan
TR-k66	LYSV	317	Garlic	Kırklareli/Babeski
TR-m90	LYSV	317	Leek	Edirne/Kesan
TR-OK64	OYDV	602	Onion	Bursa/Karacabey/Keslik
TR-O-187	OYDV	602	Garlic	Tekirdag/Suleymanpasa
TR-OKU	OYDV	602	Garlic	Kırklareli/Vize
TR-OK90	OYDV	602	Leek	Kırklareli/Luleburgaz
OYDVM82	OYDV	602	Garlic	Edirne/Kesan
OYDVM22	OYDV	602	Onion	Sakarya/Pamukova

Determination of the reaction of onion and garlic varieties to OYDV

A study was conducted in the Atatürk Horticultural Central Research Institute, Yalova-Türkiye on 6 garlic and 15 onion genotypes using pot trials and the coincidence plots experimental design (Table 2).

Table 2
Onion and garlic genotypes
used in OYDV reactions

Species	Genotype
Onion	19Y17
Onion	19Y19
Onion	19Y86
Onion	19Y51
Onion	19Y42
Onion	19Y15
Onion	19Y72
Onion	19Y65
Onion	19Y47
Onion	19Y75
Onion	19Y20
Onion	19Y79
Onion	19Y29
Onion	19Y11
Onion	Besirli
Garlic	Mugla 3
Garlic	Mugla 5
Garlic	Mugla 6
Garlic	Seleksiyon 4
Garlic	Kahramanmaras
Garlic	3.42/3

9 plants for each genotype were used in the reaction study, 3 plants used as negative control for each genotype. Virus inoculation was performed on the genotypes when they were in the 2–4 leaf stage, depending on their vegetation period. The study considered plant height, bulb mass, bulb height, and width as plant growth parameters to determine the reactions of garlic and onion genotypes. The experimental data were evaluated using the JMP Pro 13 programme.

Results

Virus incidence in fields and identification of potyviruses

Viral symptoms were observed in the samples collected from the surveyed areas, including discolouration, pitting, general yellowing, chlorotic streaks, drying starting from the leaf tips, irregular streaks parallel to the midrib, deformity of the leaves, stunting, growth retardation and shrinkage of the stem. Only plants without symptoms were selected for sampling. Observed onion leaves exhibited pitting, curling, yellowing, and partial stunting, while garlic leaves showed yellowing and garlic bulb and teeth experienced shrinking.

Table 3
Onion samples tested by serological method and found infected

Province	County	Total Number of Samples Tested	OYDV	LYSV	LYSV + OYDV
Bursa	Karacabey	120	12	3	-
	Yenisehir	50	14	1	-
	Mustafakemalpasa	30	9	-	-
Tekirdag	Malkara	60	18	6	4
	Suleymanpasa	69	11	-	-
Kocaeli	Karamursel	25	1	-	-
Sakarya	Pamukova	19	9	-	-
	Geyve	21	9	-	-
Kirkklareli	Vize	15	5	-	-
	Luleburgaz	11	-	-	-
Edirne	Kesan	15	6	3	3
Total		435	94	13	7

The onion samples were collected from Yenisehir and Mustafakemalpasa counties in Bursa province, Malkara and Suleymanpasa counties in Tekirdag province, Karamursel county in Kocaeli province, Pamukova and Geyve counties in Sakarya province, Vize and Luleburgaz counties in Kirkklareli province, and Kesan county in Edirne province. A total of 435 samples were collected and 107 samples were determined as infected. 94 were found to have OYDV, 13 had LYSV, and 7 had a mixed infection of OYDV and LYSV (Table 3).

Table 4
Leek samples tested by serological method and found infected

Province	County	Total Number of Samples Tested	OYDV	LYSV	LYSV + OYDV
Bursa	Karacabey	90	25	36	8
	Yenisehir	83	7	-	-
Kirkklareli	Luleburgaz	25	2	9	-
Edirne	Kesan	21	-	11	-
Total		219	34	56	8

Out of the 219 leek samples collected from Karacabey and Yenisehir counties in Bursa province, Luleburgaz county in Kirkklareli province, and Kesan county in Edirne province. 34 leek samples were found infected with OYDV and 56 leek samples were found infected with LYSV. Additionally, 8 of the infected leek samples showed mixed infection of LYSV and OYDV (Table 4).

Table 5
Garlic samples tested by serological method and found infected

City	District	Total Number of Samples Tested	OYDV	LYSV	LYSV + OYDV
Tekirdag	Suleymanpasa	18	2	-	-
	Malkara	18	5	3	1
Kirkklareli	Babaeski	30	14	1	1
	Vize	50	23	3	2
Edirne	Kesan	8	3	3	2
Total		124	47	10	6

OYDV was detected in 47, LYSV in 10, and LYSV + OYDV in 6 out of the 124 garlic samples collected from Suleymanpasa, Malkara, Babaeski, Vize, and Kesan counties (Table 5). Curling, pitting, leaf discoloration, mosaic like coloration on onion leaves and drying of leaf tips were observed in mechanical inoculation studies (Fig. 2). In molecular studies, 43 *Allium* samples were subjected to RT-PCR selected from the isolates (Table 6).

Table 6
Garlic samples found positive by DAS-ELISA

No	Plant	Sample Number	Location	Collection Date
1	Leek	LYSV-K84	Kırklareli/Luleburgaz/Merkez	2021
2	Leek	LYSV-P15	Bursa/Karacabey/Tophisar	2020
3	Leek	LYSV-M95	Edirne/Kesan/Sukruköy	2021
4	Leek	LYSV-M90	Edirne/Kesan/Seydiköy	2021
5	Garlic	LYSV-M82	Edirne/Kesan/Lalacık	2021
6	Leek	LYSV-M104	Edirne/Kesan/Kadıköy	2021
7	Leek	LYSV-M86	Edirne/Kesan/Seydiköy	2021
8	Garlic	LYSV-M79	Edirne/Kesan/Lalacık	2021
9	Onion	LYSV-M36	Tekirdag/Malkara/Yaylagöne	2021
10	Leek	LYSV-P17	Bursa/Karacabey/Tophisar	2020
11	Leek	LYSV-P55	Bursa/Karacabey/Kartal	2020
12	Garlic	LYSV-M186	Tekirdag/Malkara	2020
13	Garlic	LYSV-24	Kırklareli/Vize/Çavusköy	2021
14	Leek	LYSV-P35	Bursa/Karacabey/Tophisar	2020
15	Garlic	LYSV-K66	Kırklareli/Babaeski	2021
16	Onion	LYSV-M66	Tekirdag/Malkara/Halıçköy	2021
17	Leek	LYSV-56	Bursa/Karacabey/Tophisar	2020
18	Leek	LYSV-59	Bursa/Karacabey/Tophisar	2020
19	Leek	LYSV-83	Edirne/Kesan/Lalacık	2021
20	Leek	LYSV-P18	Bursa/Karacabey/Tophisar	2020
21	Onion	OYDV-TR-OK64	Bursa/Karacabey/Keslik	2021
22	Garlic	OYDV-TR-OKU	Kırklareli/Vize19202122	2021
23	Onion	OYDV-M24	Tekirdag-Malkara	2020
24	Garlic	OYDV-K59	Kırklareli/Babaeski	2021
25	Garlic	OYDV-K67	Kırklareli/Babaeski	2021
26	Garlic	OYDV-K58	Kırklareli/Vize/Çavusköy	2021
27	Garlic	OYDV-K55	Kırklareli/Vize/Çavusköy	2021
28	Onion	OYDV-45	Bursa/Karacabey/Karasu	2020
29	Onion	OYDV-72	Bursa/Karacabey/Keslik	2020
30	Onion	OYDV-118	Bursa/Yenisehir/Köprühisar	2020
31	Garlic	OYDV-K65	Kırklareli/Babaeski	2021
32	Onion	OYDV-M66	Tekirdag/Malkara/Pazardere	2020
33	Onion	OYDV-M33	Tekirdag/Malkara/Yaylagöne	2020
34	Onion	OYDV-M22	Sakarya/Pamukova/Fevziye	2020
35	Onion	OYDV-M74	Edirne-Kesan/Merkez	2021
36	Garlic	OYDV-M51	Tekirdag/Malkara/Pazardere	2021
37	Garlic	OYDV-M77	Edirne-Kesan/Merkez	2020
38	Leek	OYDV-P35	Bursa/Karacabey/Tophisar	2020
39	Garlic	OYDV-M82	Edirne/Kesan/Lalacık	2021
40	Leek	OYDV-TR-OK90	Kırklareli/Luleburgaz/Merkez	2021
41	Garlic	OYDV-TR-O-187	Tekirdag/Suleymanpasa	2020

No	Plant	Sample Number	Location	Collection Date
42	Onion	OYDV-221	Tekirdag/Malkara Yaylagöne	2020
43	Onion	OYDV-240	Sakarya/Pamukova/Bayat	2020

Agarose gel electrophoresis were conducted using RT-PCR products, and the resulting bands were visualised under UV transilluminator. Oligonucleotides of the Nib-CP gene region of *Onion yellow dwarf virus* was amplified using OYDV-F 5'-CAC CNT AYA TAG CRG ARA CAG CTC T-3' and OYDV-R 5'-ACT GAA ATG CGC CAT TAT YTG YCT A-3'. 602 bp in length; LYSV was amplified using Nib-F-5' CGC ATA TGC AGG GAT GTT TCG GTT-3' and LYSV CP-R 5'-ATC AGC ATT CAG GCT GCT GCT TAT ACA C-3' primers, approximately 317 bp long bands were obtained (Fig. 3, Fig. 4).

Phylogenetic analysis

The partial nucleotide sequences of OYDV isolates were compared with 23 isolates from around the world registered in the NCBI GenBank. The OYDV TR-OK64 (GenBank accession number: OR923732) was isolated from onions in Bursa/Karacabey, OYDV TR-O-187 (GenBank accession number: OR923731) was isolated from garlic in Tekirdag/Suleymanpasa, OYDV TR-OKU (GenBank accession number: OR923734) was isolated from garlic, and OYDV TR-OK90 (GenBank accession number: OR923733) was isolated from leek showed a similarity of 76.4–85.3% each other. OYDV sequences obtained from the GenBank showed 76.3–81.3% nt identity for OYDV TR-OK90, 75.4–85.3% nt identity for OYDV TR-O-187, 75.5–100% nt identity for OYDV TR-OK64, 74.1–97.5% nt identity for OYDV TR-OK90 (Fig. 5). The lowest identity percentages (74.1–76.3% nt) were observed with a OYDV India (GenBank accession number: KJ451436), the Netherlands (GenBank accession number: AB000836) and China (GenBank accession number: MN059593) isolate. However, all isolates from Türkiye showed the highest nucleotides identity with a United Arab Emirates Guae isolate (GenBank accession number: AB000839), China isolates (GenBank accession number: MN059561, NC_005029 and MT358349), respectively.

LYSV sequences obtained from the GenBank database (National Center for Biotechnology Information) showed 84.6–98.1% nt identity for LYSV TR-24, 86.1–97.8% nt identity for LYSV TR-k66 87.6–94.4% nt identity for LYSV TR-m82, 86.9–97.4% nt identity for LYSV TR-m90, 86.5–98.1% nt identity for LYSV TR-p55, 86.9–97.8% nt identity for LYSV TR-p17 (Fig. 6). The lowest identity percentages (84.6–87.6% nt) were observed with a LYSV Brazil (GenBank accession number: ON565071) and Spain (GenBank accession number isolate JX429965) isolate. However, all isolates from Türkiye showed the highest nt identity with a Iran isolate (GenBank accession number: MT232838) and Japan isolate (GenBank accession number: AB005612). Phylogenetic relationships of 28 LYSV sequences selected according to their geographical origin revealed that Türkiye isolates were positioned among sequences from other countries.

Garlic and onion genotypes reactions to OYDV and statistical analyses

During the study, 6 garlic genotypes were observed for discolouration, necrotic area formation, and drying of leaf tips as a reaction. The root structure of the healthy Mugla 5 genotype was found to be longer and healthier than that of the OYDV inoculated plant. The Bursa OYDV TR-OK64 isolate was propagated and used in an onion reaction study. Fifteen onion genotypes were inoculated, and the resulting symptoms included pitting of leaves, drying of leaf tips, oval-shaped yellowing, and zigzag leaf formation. The study investigated the effects of healthy onion and garlic genotypes and virus-inoculated genotypes on plant parameters. Garlic genotypes inoculated with OYDV TR-OK64 exhibited a decrease of 13.49–23.90% in leaf length, 23.21–29.14% in bulb mass, 21.88–30.81% in bulb width, and 18.38–28.73% in bulb height. The longest leaf length was observed in Mugla 3 (29.33 cm), while the shortest was observed in 3.42/3 (23.33 cm) garlic genotypes. The highest bulb mass was determined in 3.42/3 (0.52 g), whereas the lowest was determined in the Kahramanmaraş (0.30 g) genotype. Selection 4 had the largest bulb width (20.00 mm), while the smallest was found in the Kahramanmaraş genotype (11.33 mm). Selection 4 also had the longest bulb height (17.00 mm), while the shortest was found in the Mugla 5 genotype (12.33 mm) (Table 7). The inoculation of OYDV TR-OK64 onion genotypes resulted in a decrease of 12.12–29.32% in leaf length, 12.96–48.30% in bulb mass, 11.57–30.17% in bulb width, and 11.81–32.63% in bulb height. Among the control onion plants, the highest bulb mass was observed in the Besirli genotype, weighing 5.4 g, while the lowest weight was recorded in the 19Y72 genotype, weighing only 1.2 grams. The maximum bulb width was obtained in Besirli, measuring 63.00 mm, whereas the minimum bulb width was observed in 19Y86, measuring 30.67 mm. Similarly, the maximum bulb height was recorded in Besirli, measuring 69.17 mm, while the minimum bulb width was observed in 19Y06, measuring 32.67 mm (Table 8).

Table 7
Reaction data of garlic genotypes inoculated with OYDV TR-OK64

Genotype	Leaf Length (cm)			Bulb height (mm)			Bulb width(mm)			Bulb mass(g)		
	Control	Inoculation	%R ¹	Control	Inoculation	%R ¹	Control	Inoculation	%R ¹	Control	Inoculation	%R ¹
Mugla 3	29.33 ± 1.15a	22.3 ± 0.70a	23.9	12.50 ± 1.50b	9.10 ± 1.15b	27.23	16.67 ± 0.58b	13.00 ± 0.35c	22.18	0.35 ± 0.01c	0.26 ± 0.04c	24.68
Kahramanmaras	25.66 ± 0.58bc	22.00 ± 0.50a	14.28	13.33 ± 0.76b	9.50 ± 0.50b	28.73	11.33 ± 0.58d	7.83 ± 0.29e	30.81	0.30 ± 0.01d	0.21 ± 0.01d	29.14
Mugla 5	27.00 ± 1.0b	21.22 ± 1.57ab	21.21	12.33 ± 0.58b	10.06 ± 0.50b	18.38	13.67 ± 0.58c	10.43 ± 0.51d	23.64	0.41 ± 0.01b	0.31 ± 0.02 ± b	23.59
3.42/3	23.33 ± 0.58d	20.16 ± 0.58b	13.49	16.67 ± 0.58a	13.16 ± 0.76a	21.01	17.83 ± 1.04b	14.33 ± 1.04ab	21.88	0.52 ± 0.03a	0.40 ± 0.02a	23.21
Mugla 6	24.00 ± 1.00cd	20.03 ± 0.45b	16.46	16.33 ± 0.58b	12.53 ± 0.50a	23.25	18.33 ± 1.26ab	13.53 ± 0.50bc	23.99	0.36 ± 0.01bc	0.28 ± 0.02bc	23.88
Seleksiyon 4	25.66 ± 1.53bc	19.66 ± 1.53b	23.22	17.00 ± 1.15a	13.33 ± 1.00a	21.62	20.00 ± 1.73a	15.20 ± 0.76a	24	0.38 ± 0.03bc	0.28 ± 0.03bc	25.49
cv	3.97	4.82		5.09	6.07		6.07	5.10		7.73	7.28	

Percentage change between control plant and plant with virus inoculation

Each column has been evaluated within itself.

Each column was analysed in its own column. Data for each genotype were analysed as control and inoculation ($p < 0.05$). Different letters following the means in the same column indicate that the means are statistically significantly different between genotypes (Anova $p < 0.05$, LSD test). * There is a statistically significant difference between the inoculated and control treatments of the genotype in the trait analysed (Anova $p < 0.05$, t-test)

Table 8
Reaction data of onion genotypes inoculated with OYDV TR-OK64

Leaf Length (cm)				Bulb height (mm)			Bulb width(mm)			Bulb mass(g)		
Genotype	Control	Inoculation	%R ¹	Control	Inoculation	%R ¹	Control	Inoculation	%R ¹	Control	Inoculation	%R ¹
19Y17	46.33 ± 1.52h	36.33 ± 2.08f	21.58	45.67 ± 1.52gh	36.83 ± 0.76f	19.36	44.00 ± 1.00de	35.10 ± 0.17de	20.23	2.7 ± 0.028e	1.85 ± 0.50g	31.48
19Y15	33.67 ± 1.72j	26.67 ± 0.76hi	20.79	41.33 ± 0.57i	32.83 ± 1.04g	20.57	39.00 ± 1.73g	30.67 ± 1.04f	21.36	2.33 ± 0.11f	1.48 ± 0.76h	36.48
19Y142	28.67 ± 1.65k	23.07 ± 0.50j	19.53	40.67 ± 0.67i	31.30 ± 0.30h	23.04	42.67 ± 3.21ef	34.20 ± 0.30de	19.85	2.1 ± 0.05g	1.38 ± 0.29h	34.29
19Y72	37.50 ± 0.70i	30.50 ± 1.32g	18.67	40.33 ± 0.59i	27.17 ± 1.24i	32.63	35.33 ± 0.59h	24.67 ± 1.24g	30.17	1.2 ± 0.18j	0.75 ± 0.50j	37.50
19Y06	34.67 ± 2.30ij	25.00 ± 2.00ij	27.89	32.67 ± 1.15j	25.67 ± 0.58j	21.43	32.67 ± 1.54ij	26.33 ± 0.58g	19.41	1.36 ± 0.06ij	1.03 ± 1.04i	24.26
19Y19	36.67 ± 1.15ij	28.50 ± 1.50gh	22.28	44.67 ± 0.87h	30.50 ± 0.71h	31.72	34.67 ± 0.57hi	25.17 ± 1.89g	27.40	1.76 ± 0.15h	1.11 ± 1.24i	36.93
19Y86	45.00 ± 1.73h	34.83 ± 2.47f	22.60	41.00 ± 1.00i	31.25 ± 1.32h	23.78	30.67 ± 2.08j	24.50 ± 1.80g	20.12	1.53 ± 0.17i	1.05 ± 0.60i	31.37
19Y47	59.00 ± 1.96de	43.33 ± 2.02de	26.56	59.33 ± 1.18c	41.00 ± 1.00d	30.89	52.33 ± 1.16b	41.42 ± 2.92b	20.85	4.7 ± 0.23b	2.43 ± 2.08e	48.30
19Y20	63.00 ± 1.36c	44.53 ± 1.50de	29.32	63.00 ± 0.65b	44.53 ± 1.36b	29.32	48.67 ± 0.67c	38.53 ± 1.75c	20.83	4.36 ± 0.02c	3.18 ± 0.76c	27.06
19Y75	66.67 ± 3.05b	57.67 ± 2.52b	13.50	46.67 ± 0.58g	36.50 ± 0.50f	21.79	40.67 ± 0.57fg	32.67 ± 0.58ef	19.67	3.51 ± 0.1d	2.11 ± 0.29g	39.89
19Y79	56.67 ± 1.52ef	44.87 ± 2.50d	20.82	40.69 ± 1.64i	31.87 ± 0.81gh	21.68	43.00 ± 1.00de	35.00 ± 1.00de	18.60	2.9 ± 0.12e	2.26 ± 0.58f	22.07
19y29	52.00 ± 3.46g	41.73 ± 0.81e	19.75	49.67 ± 1.52f	39.50 ± 0.58e	20.48	45.00 ± 1.73d	35.33 ± 1.53d	21.49	3.33 ± 0.16d	2.67 ± 1.53d	19.82
19Y11	53.67 ± 2.88fg	41.83 ± 1.04de	22.06	54.67 ± 1.18e	42.50 ± 0.60c	22.26	48.17 ± 0.28c	38.67 ± 1.15c	19.72	4.33 ± 0.05c	3.50 ± 1.00b	19.17
19Y65	60.67 ± 1.15cd	51.33 ± 2.75c	15.39	57.33 ± 0.57d	45.83 ± 1.04b	20.06	47.67 ± 0.57c	39.00 ± 1.00bc	18.19	4.23 ± 0.17c	3.45 ± 0.87b	18.44
Besirli	75.67 ± 1.54a	66.50 ± 1.50a	12.12	69.17 ± 0.28a	61.00 ± 1.00a	11.81	63.00 ± 0.58a	56.00 ± 1.20a	11.57	5.4 ± 0.11a	4.7 ± 1.10a	12.96
cv	3.85	4.6		1.8	2.38		3.12	4.29		4.09	4.39	

¹Percentage change between control plant and plant with virus inoculation

Each column has been evaluated within itself.

Each column was analysed in its own column. Data for each genotype were analysed as control and inoculation ($p < 0.05$). Different letters following the means in the same column indicate that the means are statistically significantly different between genotypes (Anova $p < 0.05$, LSD test). * There is a statistically significant difference between the inoculated and control treatments of the genotype in the trait analysed (Anova $p < 0.05$, t-test)

Many plants in the same field were found infected with the same virus, which spread rapidly in the area. Obtaining healthy production material was deemed more important than increasing the number of garlic plants. As garlic growers often re-use production materials from year to year, it may be assumed that the producer is not analysing the materials closely enough. This can lead to favourable conditions for the spread of viruses, ultimately resulting in increased yield loss. It is important to note that viruses can easily be transported over long distances, making the use of certified production materials crucial. Weeds that host viruses can spread infections and pose a threat to production. Therefore, measures should be taken against both weeds and vectors that carry viruses. This study determined the presence of IYSV in Alliums for the first time in our country. Reaction studies revealed that viruses affect development parameters. The production of garlic in Serbia is significantly impacted by a number of diseases caused by viruses, with the most economically significant being the OYDV. Infected plants showed a decrease in height, mass, and dry matter content compared to healthy plants. The study reported a decrease of 21.5% in bulb mass and 24.0% in dry matter yield per bulb content (Bagi et al. 2012). In our study included mechanical inoculation of OYDV TR-OK64 to garlic genotypes, and the decrease in mass was found to be between 23.21–29.14%, which is consistent this study conducted in Serbia. Additionally, reaction studies revealed loss due to developmental retardation, which is a common consequence of virus diseases. Successful inoculation of OYDV can be achieved by using infected green leaves. This can be achieved after several inoculations (Louie and Lobeer, cited in Massoud and Abo-Elela, 2008). In our study, we observed that the leaves of the Giza 6 onion variety, which were inoculated with OYDV, exhibited symptoms such as curling, yellow banding, spots, and stunting. In onion and garlic fields, it was observed that OYDV-infected plants resulted in a significant decrease in stem length, number of leaves, plant weight, onion weight, as well as the number and weight of garlic cloves. Green parts of the onion used in the inoculation study and achieved success in the reaction study with the second inoculation method after the first inoculation (Elnagar et al. 2011).

Discussion

OYDV and LYSV were detected viral pathogens in onions, garlic, and leeks grown in Marmara Region, Türkiye. OYDV as the most prevalent virus with garlic having the highest incidence rate of 44.4% (55/124), followed by onion at 34.6% (9/26), and leek at 5.4% (7/129) in the Czech Republic (Mansouri et al. 2021). In our research OYDV was reported most frequently virus on onion, garlic and leek. The infection rates were found to be 21.6% (94/435) in onion, 37.9% (47/124) in garlic and leek 15.52% (34/219). LYSV was high infection rates on leek 25.57% (56/219), following garlic %8.06 (10/124) and onion %2.98 (13/435). The viruses detected in garlic were reported to be introduced through imported production material from Iran and China (Dovas et al. 2001). It is believed that the use of garlic, grown in different regions of our country, as a production material without analysis is highly effective in transferring the virus from one place to another. In similar studies conducted in our country, 12.33% of onion production areas in Amasya province were found to be infected with OYDV, 3.60% with LYSV and 1.19% with SLV (Sevik and Akcura 2012). In this study, OYDV isolates obtained from 6 garlic, 2 onion, and 4 leek plants Marmara Region were amplified using primers specific to *Allium* potyviruses (LYSV, OYDV) to encode the CP gene.

The phylogenetic tree showed that OYDV isolates were grouped with garlic isolates, while onion and leek isolates were in separate groups. The high similarity of garlic isolates may be attributed to their shared species and close sampling sites. The LYSV and OYDV isolates were classified into two groups: wakegi onion and garlic. The garlic isolates were found to be in the same group, while the wakegi onion isolates were in a separate group. The phylogenetic analysis of LYSV isolates showed that leek isolates were in the same group, while two of the garlic isolates were in the same group and one was in a different group from the other garlic isolates (Tsuneyoshi et al. 1998). The LYSV isolates were classified into three groups based on their origin: leek isolates, garlic isolates from northern Japan, and garlic isolates from China, Korea, and southern Japan. This classification was determined through analysis of the 5'-UTR nucleotide and P1 amino acid sequences (Takaki et al. 2005). LYSV TR-m90, LYSV TR-p17, and LYSV TR-p55 leek isolates were grouped together, while LYSV TR-24 and LYSV TR-k66 garlic isolates were grouped together. LYSV TR-m82 garlic isolate was in a separate group. This suggests that the garlic isolates were infected with two different LYSV species. Two garlic isolates were found to be infected with different LYSV species; one belonging to the Chinese group and the other to the northern Japanese group.

In this study results from indicate the significance of OYDV a prevalent viral pathogen on onion and garlic as well as LYSV widespread on leek, in Türkiye Marmara region. As a result of the OYDV inoculation, there was a significant loss of yield in the onion and garlic genotypes due to OYDV infection.

Declarations

Data Availability The datasets utilised and/or analysed during the current study are available from the corresponding author upon reasonable request.

Acknowledgements The Republic of Türkiye, the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (TAGEM) is acknowledged for the financial support.

Funding

Financial support (Project no: TAGEM/BSAD/A/21/A2/P1/2483).

Author information

Authors and Affiliations

Department of Plant Protection, Atatürk Horticultural Central Research Institute, 77100 Yalova, Türkiye

Fatma Şafak

Department of Plant Protection, Faculty of Agriculture, Tekirdağ Namık Kemal University, 59030 Tekirdağ, Türkiye

Gassan Köklü

Author contributions The article was prepared from the Ph.D degree thesis (YOK Thesis No: 845529) of FS who designed the study, carried out the experiments and wrote the original draft. GK helped in reviewing the final draft.

Research involving This article was produced without the use of humans or animals by authors.

Conflict of interest The authors confirm that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Abubaker M.Y.A, Gabbani MFMA, Elhassan S.M (2019). Occurrence and influence of Sudanese *Onion yellow dwarf potyvirus* isolates on the common bulbing onion (*Allium cepa* L.). *Asian Research Journal of Agriculture*, 10(3), 1-18. <https://doi.org/10.9734/arja/2018/45761>
2. Arya M, Baranwal V.K, Ahlawat Y.S, Singh L (2006). RT-PCR detection and molecular characterization of *Onion yellow dwarf virus* associated with garlic and onion. *Current Science*, 91(9), 1230-1234. <https://www.jstor.org/stable/24094103>

3. Ayed C, Marais A, Faure C, Candresse T, Hamdi I, Najar A, Dridi B (2019). First report of *Onion yellow dwarf virus*, *Leek yellow stripe virus*, and *Shallot latent virus* on garlic (*Allium sativum*) in Tunisia. *Plant Disease*, 103(8), 2143-2144. <https://doi.org/10.1094/PDIS-09-18-1618-PDN>
4. Bagi F, Stojscaron V, Budakov D, El Swaeh S.M, Gvozdanovic-Varga J (2012). Effect of *Onion yellow dwarf virus* (OYDV) on yield components of fall garlic (*Allium sativum* L.) in Serbia. *African Journal of Agricultural Research*, 7(15), 2386-2390. <https://doi.org/10.5897/AJAR11.1772>
5. Bos L (1976). *Onion yellow dwarf virus*. CMI/AAB Descriptions of Plant Viruses, 158(4).
6. Bos L, Huijberts N, Huttinga H, Maat D.Z (1978). *Leek yellow stripe virus* and its relationships to *Onion yellow dwarf virus*; characterization, ecology and possible control. *Netherlands Journal of Plant Pathology*, 84, 185-204. <https://doi.org/10.1007/BF02650386>
7. Chen J, Adams M.J (2001). Molecular characterization of a complex mixture of viruses in garlic with mosaic symptoms in China. *Archives of Virology*, 146(10), 1841-1853.
8. Conci V.C, Canavelli A, Lunello P, Di Rienzo J, Nome S.F, Zumelzu G, Italia R (2003). Yield losses associated with virus-infected garlic plants during five successive years. *Plant Disease*, 87(12), 1411-1415. <https://doi.org/10.1094/PDIS.2003.87.12.1411>
9. Cremer J, Campbell P, Steele V, Persley D, Thomas J, Harper S, Gambley C (2021). Detection and Distribution of Viruses Infecting Garlic Crops in Australia. *Plants*, 10(5), 1013. <https://doi.org/10.3390/plants10051013>
10. Diekmann M (1997). *Food and Agriculture Organization; International Plant Genetic Resources Institute; Allium spp.* FAO / IPGRI Technical Guidelines for the Safe Movement of Germplasm n.18 60 p. ISBN: 978-92-9043-346-0.
11. Dovas C.I, Hatziloukas E, Salomon R, Barg E, Shibolet Y, Katis N.I (2001). Incidence of viruses infecting *Allium* spp. in Greece. *European Journal of Plant Pathology*, 107(7), 677-684. <https://doi.org/10.1023/a:1011958914573>
12. Elnagar S, El-Sheikh M.A.K, Abd El-Wahab A.S.E (2011). Effect of natural infection with *Onion yellow dwarf virus* (OYDV) on yield of onion and garlic crops in Egypt. *Journal of Life Sciences*, 5, 634-638.
13. Entezari A, Mehrvar M, Zakiaghi M (2021). Identification of garlic-infecting *Leek yellow stripe virus* through deep-sequencing analyses from Iran. *Virus Disease*, 32(3), 595-600. <https://doi.org/10.1007/s13337-021-00733-z>
14. Fernandez-Tabanera E, Fraile A, Lunello P, García-Arenal F, Ayllon M.A (2018). First Report of *Onion yellow dwarf virus* in Leek (*Allium ampeloprasum* var. *porrum*) in Spain. *Plant Disease*, 102(1), 256-256. <https://doi.org/10.1094/PDIS-06-17-0892-PDN>
15. Galmarini C. R (2018). Economic and Academic Importance. *The Allium Genomes*, 1–9. doi:10.1007/978-3-319-95825-5_1. https://doi.org/10.1007/978-3-319-95825-5_1
16. Gilani S.T, Hameed S, Sha, H (2016). Occurrence and distribution of potyviruses infecting garlic in Pakistan. *Pakistan Journal of Agricultural Research*, 29(1), 97-102.
17. Gupta N, Jain R.K, Rao G.P, Baranwal V.K (2017). Molecular characterization and phylogenetic analysis of coat protein gene of *Leek yellow stripe virus* infecting garlic in India. *Indian Phytopathology* 70(1), 114-121. <https://doi.org/10.24838/ip.2017.v70.i1.48991>
18. Harti H, Hidayat S.H, Wiyono S (2020). Detection of major viruses infecting shallot and molecular characterization of *Onion yellow dwarf virus* from several locations in Indonesia. *Biodiversitas Journal of Biological Diversity*, 21(4), 1697-1701. <https://doi.org/10.13057/biodiv/d210451>
19. Kebede Y., Singh J, Majumder, S (2020). Molecular characterization of the partial coat protein gene of an *Onion yellow dwarf virus* isolate detected in garlic (*Allium sativum* L.) from the West Shewa zone of Ethiopia. *Journal of Plant Protection Research*, 60(1). <https://doi.org/10.24425/jppr.2020.132205>
20. Kluckackova J, Navratil M, Duchoslav M (2007). Natural infection of garlic (*Allium sativum* L.) by viruses in the Czech Republic. *Journal of Plant Diseases and Protection*, 114(3), 97-100. <https://doi.org/10.1007/BF03356714>
21. Lot H, Chovelon V, Souche S, Delecalle B (1998). Effects of *Onion yellow dwarf* and *Leek yellow stripe* viruses on symptomatology and yield loss of three French garlic cultivars. *Plant Disease*, 82(12), 1381-1385. <https://doi.org/10.1094/PDIS.1998.82.12.1381>
22. Louie R, Lorbeer J.W (1966). Mechanical transmission of *Onion yellow dwarf virus*. *Phytopathology* 56, 1020-1023.
23. Mangilli A, Mohammed H. S, El Hussein A. A, Agosteo G. E, Albanese G, Tomassoli L (2014). Molecular analysis of the 3'terminal region of *Onion yellow dwarf virus* from onion in southern Italy. *Phytopathologia Mediterranea*, 53(3), 438-450. https://doi.org/10.14601/Phytopathol_Mediterr-14027
24. Mansouri F, Krahulec F, Duchoslav M, Rysanek P (2021). Newly identified host range of viruses infecting species of the genus *Allium* and their distribution in six habitats in the Czech Republic. *Plant Pathology*, 70(6), 1496-1507. <https://doi.org/10.1111/ppa.13391>
25. Massoud I.L, Abo-Elela A.A (2008). Improved Techniques for The Purification and Serology of an Egyptian Isolate of *Onion yellow dwarf virus* (OYDV). *The Egyptian Journal of Experimental Biology (Botany)*, 4, 71-71.
26. Nam M, Lee Y, Park C.H, Lee M, Bae Y, Lim Y.H, Lee J.H, Moon J.S, Lee S.H (2015). Development of multiplex RT-PCR for simultaneous detection of garlic viruses and the incidence of garlic viral disease in garlic genetic resources. *The Plant Pathology Journal*, 31(1), 90-96. <https://doi.org/10.5423/PPJ.NT.10.2014.0114>
27. Oommen S, Anto R.J, Srinivas G, Karunakaran D (2004). Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. *European Journal of Pharmacology*, 485(1-3), 97-103. <https://doi.org/10.1016/j.ejphar.2003.11.059>
28. Shahraeen N, Lesemann D.E, Ghotbi T (2008). Survey for viruses infecting onion, garlic and leek crops in Iran. *Eppo Bulletin*, 38(1), 131-135. <https://doi.org/10.1111/j.1365-2338.2008.01198.x>
29. Sherevera K, Shevchenko T, Snihur H, Budzanivska I, Shevchenko O (2019). First report of *Leek yellow stripe virus* on *Allium sativum* L. in Ukraine. *Poljoprivreda i Sumarstvo*, 65(4), 47-53. <https://doi.org/10.17707/AgricultForest.65.4.05>
30. Sevik M.A, Akcura C (2012). Viruses occurring in onion crop in Amasya province, the major onion producing region in Türkiye. *Indian Journal of Virology*, 24(1), 78-81. <https://doi.org/10.1007/s13337-012-0114-z>

31. Takaki F, Sano T, Yamashita K, Fujita T, Ueda K, Kato T (2005). Complete nucleotide sequences of attenuated and severe isolates of *Leek yellow stripe virus* from garlic in northern Japan: Identification of three distinct virus types in garlic and leek world-wide. *Archives of Virology*, 150, 1135-1149. <https://doi.org/10.1007/s00705-004-0482-9>
32. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725-2729. <https://doi.org/10.1093/molbev/mst197>
33. Tsuneyoshi T, Matsumi T, Deng T.C, Sako I, Sumi S (1998). Differentiation of *Allium* carlaviruses isolated from different parts of the world based on the viral coat protein sequence. *Archives of Virology*, 143(6), 1093-1107. <https://doi.org/10.1007/s007050050358>
34. Voncina D, Curic K, Fabek S, Toth N (2016). First report of *Onion yellow dwarf virus*, *Leek yellow stripe virus*, and Garlic common latent virus on garlic in Croatia. *Plant Disease*, 100(3), 656. <https://doi.org/10.1094/PDIS-08-15-0952-PDN>
35. Vucurovic I, Nikolic D, Radovic N, Vucurovic A, Ristic D, Krstic B, Stankovic I (2017). Incidence and distribution of *Leek yellow stripe virus* in *Allium* crops in Serbia. *Pesticidi i Fitomedicina*, 32(3-4), 145-155. <https://doi.org/10.2298/PIF1704145V>
36. Winiarczyk K, Solarska E, Sienkiewicz W (2014). Prevalence of infections with *Onion yellow dwarf virus*, *Leek yellow stripe virus* and Garlic common latent virus in plants from the genus *Allium*. *Acta Scientiarum Polonorum Hortorum Cultus*, 13(3), 123-133.
37. Yılmaz N, Fidan H (2021). Collection of and serological and molecular viral disease detection in local garlic (*Allium sativum* L.) accessions collected from Northern Cyprus. *Fresenius Environmental Bulletin*, 30(1), 569-576.

Figures



Figure 1

Sample collected locations in Marmara Region, Türkiye



Figure 2
Mechanically inoculated onion plant with OYDV symptoms

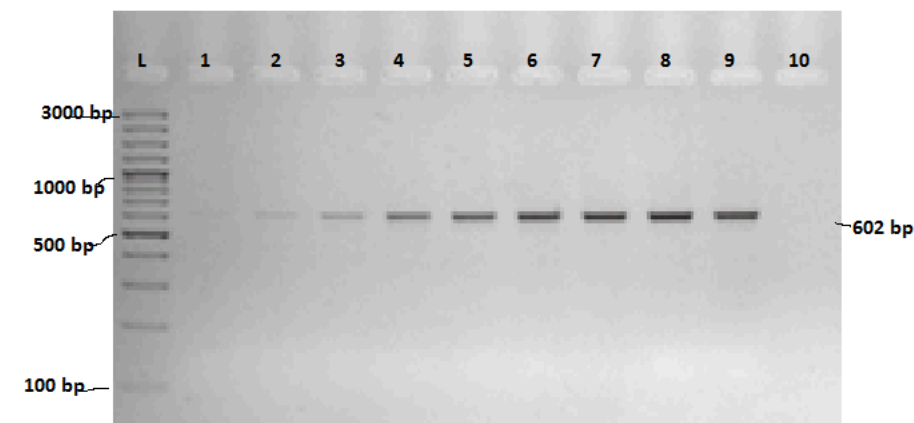


Figure 3
The appearance of the RT PCR amplifications performed using specific primers of OYDV on 1.5% agarose gel (L. GeneRuler 100 bp Plus DNA ladder, 1. OYDV-TR-OK64, 2. OYDV-TR-OKU, 3. OYDV-P35, 4. OYDV-TR-OK90, 5. OYDV-TR-OK90, 6. OYDV-TR-O-187, 7. OYDV-M51, 8. OYDV-M51, 9. OYDV-M82, 10. Negative Control).

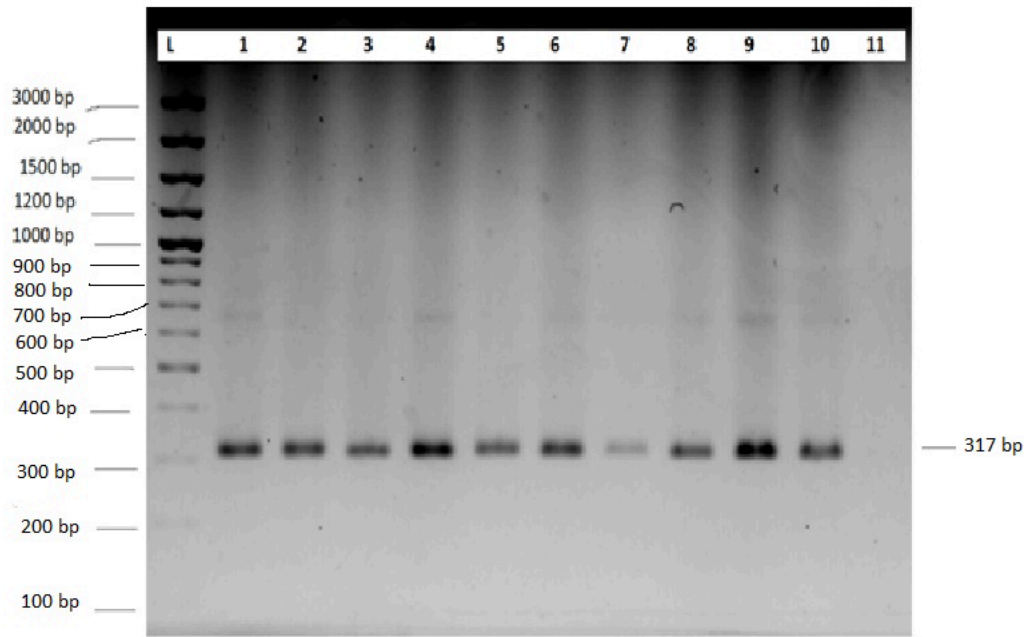


Figure 4

The appearance of RT PCR amplifications performed using specific primers of LYSV on 1.5% agarose gel (L. GeneRuler 100 bp Plus DNA ladder, 1. TR-p17, 2. LYSV-M36, 3. TR-p55, 4. TR-m82, 5. TR-k66, 6. TR-m90, 7. LYSV-K84, 8. LYSV-M95, 9. TR-24, 10. LYSV-M66, 11. Negative Control).

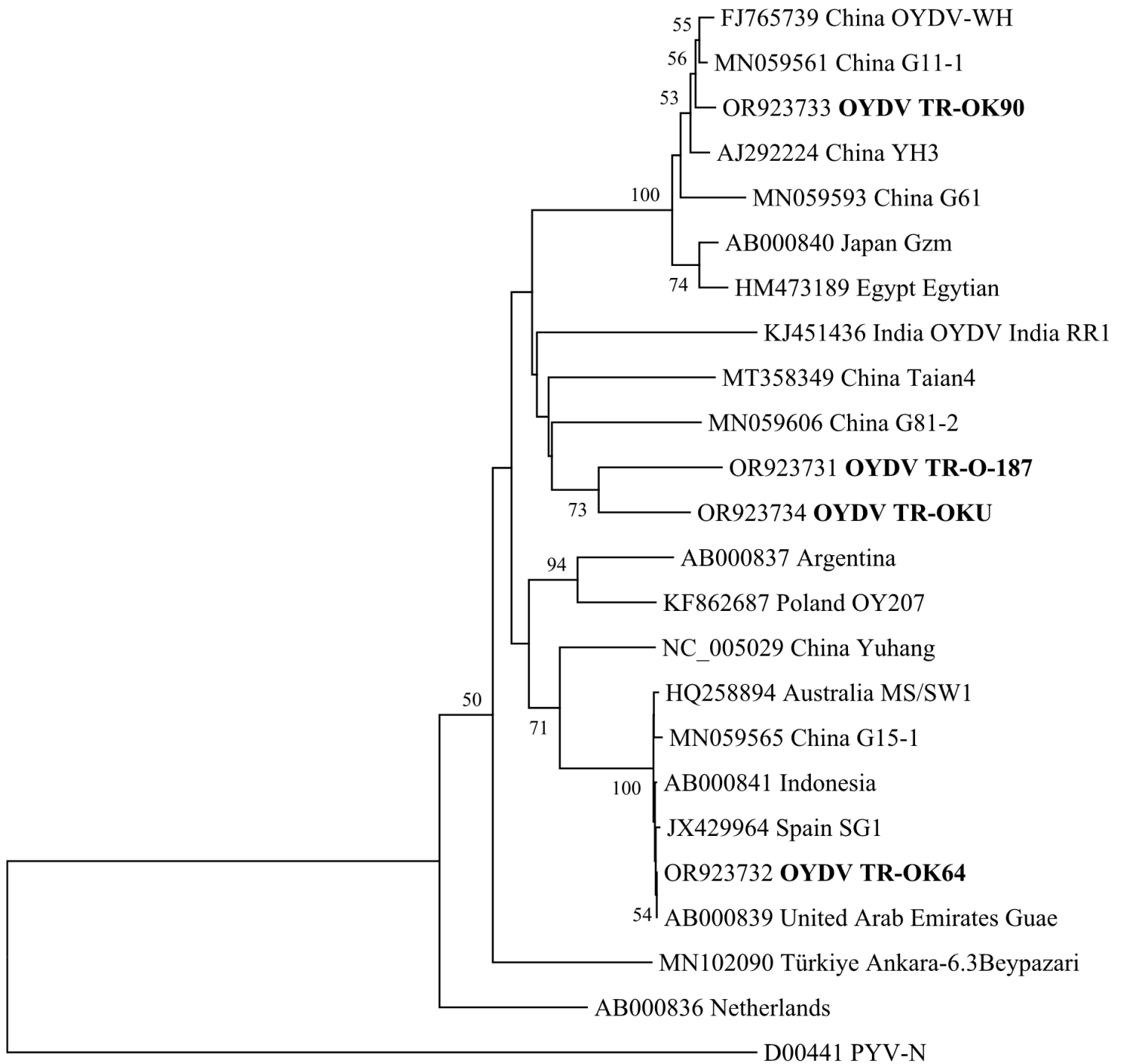


Figure 5

552 nt nucleotide sequence comparison of OYDV isolates (TR-O-187, TR-OK64, TR-OK90, TR-OKU) with 19 OYDV isolates registered in GenBank (D00441 PVY-N outgroup) (Bootstrap value 1000)

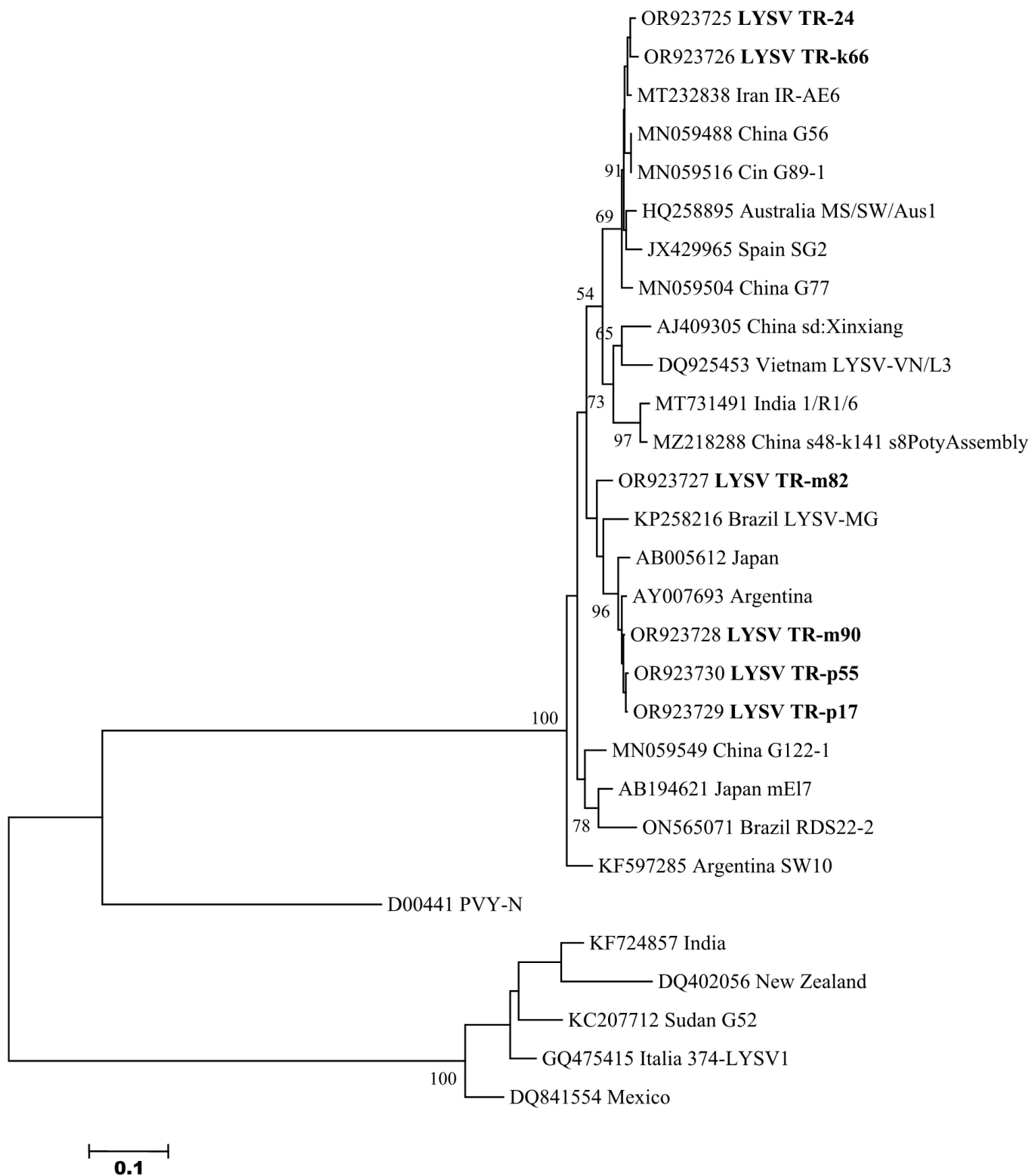


Figure 6

267 nt nucleotide sequence comparison of 6 LYSV TR isolates (LYSV TR-24, LYSV TR-k66, LYSV TR-m82, LYSV TR-m90, LYSV TR-p17 and LYSV TR-p55) with 22 LYSV isolates registered in GenBank (D00441 PVY-N outgroup) (Bootstrap value 1000)