3 Biotech (2021) 11:516
https://doi.org/10.1007/s13205-021-03067-1

ORIGINAL ARTICLE

Occurrence of leek yellow stripe virus and onion yellow dwarf virus from edible *Allium* plants in the south Marmara region of Turkey

Hasan Tuna Tuzlali[1] · **Ali Karanfil**[2] · **Savaş Korkmaz**[2]

Received: 21 May 2021 / Accepted: 15 November 2021 / Published online: 1 December 2021

Abstract

Among edible *Allium* plants (leek, onion, and garlic), leek yellow stripe (LYSV) and onion yellow dwarf virus (OYDV) are the most common viruses worldwide. While the presence of these two viruses in Turkey has previously been confirmed, only a few studies on their prevalence and genetic diversity have been performed. The aim of this study, conducted in the southern Marmara region of Turkey (SMR), was to identify the presence and genetic diversity of these viruses. Samples were collected from 494 plants exhibiting virus and virus-like symptoms. Samples were tested for the relevant viruses by double-antibody sandwich enzyme-linked immunosorbent assay and reverse transcription polymerase chain reaction (DAS-ELISA and RT-PCR, respectively). Tests revealed the presence of OYDV in 95 samples and LYSV in 52, whereas 33 samples were observed to have a combined infection. To examine the genetic diversity, 10 isolates from each virus were chosen from the infected samples. Using RT-PCR, the complete coat protein (CP) gene for LYSV and a partial sequence region of the nuclear inclusion b + CP gene for OYDV were amplified, cloned, and sequenced from the selected isolates. The sequence data were compared with the isolates in GenBank; it was determined that SMR LYSV and OYDV isolates show similarities over 77% with world isolates at the nucleotide and amino acid levels. Phylogenetic analyses showed that the LYSV and OYDV isolates had some diversity with isolates from different parts of the world, and the host had an important role in the phylogenetic relationships, particularly for LYSV.

Keywords Virus · RT-PCR · Cloning · Similarity · Phylogenetic

Introduction

Plants of the *Allium* genus are cultivated in many parts of the world for both food and medicinal purposes (Rahman and Lowe 2006). Leek, garlic, and onion stand out as the most important species belonging to this genus (Li et al. 2010).

Phytopathogens are prominent in *Allium*-type plants, as pathogens commonly affect all cultivated agricultural products. Among these phytopathogens, viruses are of particular importance because they do not respond to chemical control and cause significant yield losses. Studies conducted worldwide have reported frequent infections of potyvirus species in addition to infections caused by tospovirus, carlavirus, and allexivirus species in leek, garlic, and onion cultivation (Dovas et al. 2001; Katis et al. 2012; Taglienti et al. 2018; Abraham et al. 2019).

Members of the potyvirus genus cause significant losses among plants of the *Allium* genus (Lot et al. 1998). Leek yellow stripe and onion yellow dwarf viruses (LYSV and OYDV, respectively), the two viruses first characterized among this group of viruses, have been reported to be frequently observed in areas in which these plants are cultivated (Winiarczyk et al. 2014; Fernández-Tabanera et al. 2018; Abraham et al. 2019). Therefore, LYSV and OYDV are the two most important plant pathogens of the potyvirus genus, causing economic losses in plants of the *Allium* genus.

In many worldwide studies, LYSV and OYDV infections have been reported from different countries, and detailed analyses have been conducted concerning their genetic

✉ Savaş Korkmaz
skorkmaz@comu.edu.tr

Hasan Tuna Tuzlali
tunatuzlali@comu.edu.tr

Ali Karanfil
alikaranfil@comu.edu.tr

[1] Mersin Directorate of Agricultural Quarantine, Ministry of Agriculture and Forestry, Mersin, Turkey

[2] Department of Plant Protection, Faculty of Agriculture, Canakkale Onsekiz Mart University, Canakkale, Turkey

diversity (Dovas et al. 2001; Fajardo et al. 2001; Bereda et al. 2015). However, the number of studies from Turkey that have investigated potyvirus infection in plants of the *Allium* genus is limited, and these studies have focused on identifying only LYSV and OYDV infections (Korkmaz and Cevik 2009; Fidan and Baloglu 2009; Santosa and Ertunc 2020). In addition, the Çanakkale, Balıkesir, and Bursa provinces that constitute the southern Marmara region of Turkey (SMR) are among the leading provinces in Turkey involved in the cultivation of onion and garlic, and literature reviews have revealed that no study has been conducted to determine the prevalence and genetic diversity of these potyviruses in these provinces. In this context, no study addressing the genetic diversity of SMR LYSV and OYDV isolates can be found. For these reasons, whether LYSV and OYDV infections are related to potyviruses was investigated by conducting surveys in commercial agricultural areas of SMR in which *Allium* species are cultivated. Molecular characterization of the isolates from the infected samples was performed, and their genetic diversity was investigated. This paper presents the most comprehensive study performed in Turkey to date.

Materials and methods

Sampling

Field studies were carried out in Bursa, Balıkesir, and the Çanakkale provinces of SMR in which *Allium* plants are cultivated. The survey areas were selected based on the farmer registration system data obtained from the district directorates of agriculture, the directions of the cultivators, and the opinions of the technical staff working in the relevant areas. Only samples from garlic, leek, and onion plants displaying virus and virus-like symptoms were collected based on the results from surveys conducted during the 2014 to 2017 production seasons as part of field studies.

Virus identification

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) tests were utilized to investigate OYDV and LYSV infections in the study samples. Tests were carried out with polyclonal antibodies specific to the target viruses. The tests were carried out based on the method reported by Clark and Adams (1977) in line with the recommendations of Bioreba (Switzerland) and Agdia (United States), which supplied the kits.

Furthermore, since both viruses were of the potyvirus genus, all positive samples were tested with specific primer pairs via reverse transcription-polymerase chain reaction (RT-PCR) to identify possible cross-reactions between them or with other potyviruses. First, total RNA was isolated from infected samples using the RNeasy Mini Kit (Qiagen, Canada). Complementary DNA (cDNA) was synthesized from the isolated total RNAs using PrimeScript cDNA synthesis kits (Takara, Japan). With the resulting cDNAs, tests were performed using the primer pair of 5 'TCA CTG CAT ATG CGC ACC AT 3' and 3 'GCA CCA TAC AGT GAA TTG AG 5' (1030 bp) sequences involving the complete coat protein (CP) gene and a part of the nuclear inclusion b (NIb) region, which were designed by Fajardo et al. (2001) for LYSV. The primers 5 'CAC CNT AYA TAG CRG ARA CAG CTC T 3' and 3'ACT GAA ATG CGC CAT TAT YTG YCT A 5' to amplify 602 bp of the NIb + CP region (Lee et al. 2005) for OYDV was also used.

Cloning and sequencing

Among the isolates obtained by the DAS-ELISA and RT-PCR tests, a total of 10 LYSV and 10 OYDV isolates were selected after considering the geographical origin and host from which they were obtained. CP-based molecular characterization was performed for the selected isolates to identify their genetic diversity.

In RT-PCR studies, 1030 bp amplicons of the complete CP gene and 602 bp amplicons of a part of the NIb + CP gene were obtained for LYSV and OYDV, respectively. These amplified target gene regions were cloned using pGEM®-T Easy Vector Systems (Promega, USA) by the TA cloning method, and the plasmid obtained for each isolate was sequenced bi-directionally with the primers M13F and M13R to confirm its identity.

Bioinformatics analyses

The raw sequence data obtained from sequencing were combined in CLC Main Workbench (V.7.7.3) software, and consensus sequences were deposited in NCBI GenBank (Supp. Table 1). The sequences of the world LYSV and OYDV isolates were taken from the GenBank database (Supp. Table 2 and 3). Similarities and phylogenetic relationships among the LYSV and OYDV Turkish isolates and their similarities and phylogenetic relationships with the world isolates were analyzed. The similarity rates were determined with Clustal W sequencing utilizing the sequence demarcation tool (SDT) V.1.2 (Muhire et al. 2014). Phylogenetic relationships of the isolates were investigated in MEGA 7 software with 1000 bootstrap values using the maximum likelihood method and Kimura 2 (K2) parameter model (Kumar et al. 2016).

Results

A total of 494 leek, garlic, and onion samples were collected during the field studies. Virus identification studies performed with the DAS-ELISA test found LYSV in 52 samples, OYDV in 95 samples, and combined infection of LYSV + OYDV in 33 samples (Table 1). Among the LYSV infections, 48 were observed in leek, three in onion, and one in garlic, whereas OYDV infections were identified in 18 leeks and 77 garlic plants. Twenty-one and 12 of the combined infections of LYSV and OYDV were in leek and garlic samples, respectively.

Based on the DAS-ELISA test results, OYDV infection alone was identified in five onion samples, while mixed infection of LYSV + OYDV was present in two onion samples. However, no OYDV infection was identified among these samples by the RT-PCR tests. These results showed that 180 of the collected samples were infected with at least one virus in question based on the combination of DAS-ELISA and the RT-PCR tests (Table 1).

The most typical symptom seen in samples infected with LYSV was a yellow stripe on the leaves (Fig. 1a, b). Leaf yellowing and curling were observed in onions infected with LYSV (Fig. 1c, d), whereas garlic samples infected with OYDV had mosaics on their leaves, and some plants showed a slight leaf curl (Fig. 1e, f).

After applying multiple sequence analyses to determine the genetic diversity of the LYSV isolates, the isolates were observed to exhibit a sequence similarity of 79% to 99% and 80% to 100% at the nucleotide and amino acid (nt and aa, respectively) levels. The similarity rates of these isolates when compared with world isolates were 78%–98% and 78%–100% at the nt and aa levels, respectively (Fig. 2 and Supp. Fig. 1).

The OYDV isolates were observed to exhibit a similarity of 76% to 99% and 83% to 100% at the nt and aa levels, respectively. The similarity rates of these isolates with world isolates were 77%–87% and 79%–94% at the nt and aa levels, respectively (Fig. 3 and Supp. Fig. 2).

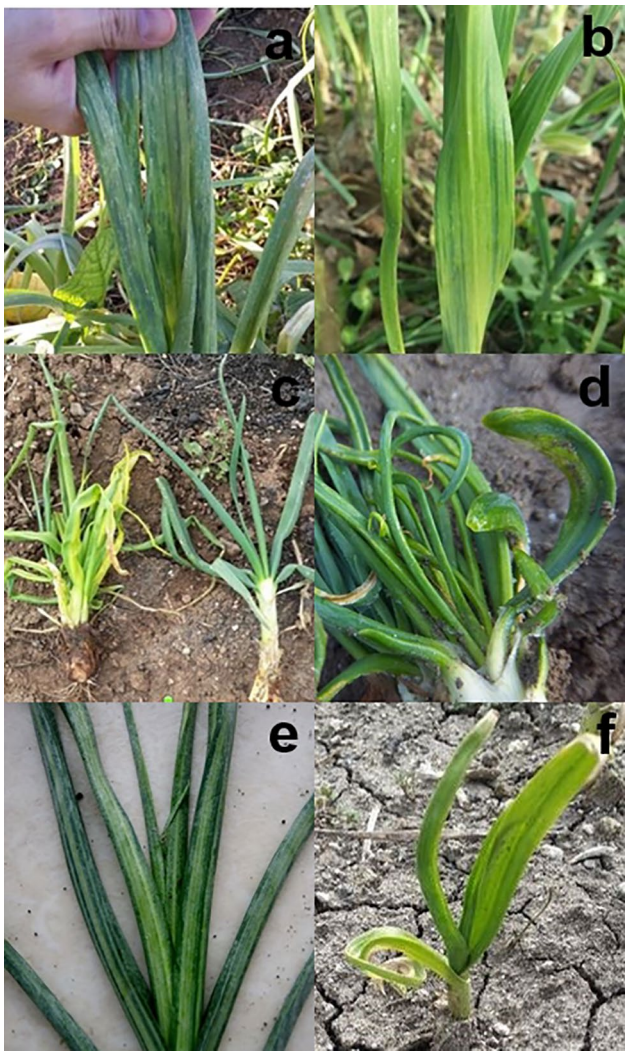

Fig. 1 Some symptoms related with leek yellow stripe virus (LYSV) and onion yellow dwarf virus (OYDV) based on the virus identification analyses (**a**, **b**: yellow stripe symptoms on leek plants infected with LYSV, **c**, **d**: leaf yellowing and curling on onion plants infected with LYSV, **e**, **f**: mosaic and mild curling symptoms on garlic plants infected with OYDV)

Table 1 Result of virus identification tests based on the combining of DAS-ELISA and RT-PCR tests

Virus		LYSV			OYDV			LYSV + OYDV		
Province		Çanakkale	Balıkesir	Bursa	Çanakkale	Balıkesir	Bursa	Çanakkale	Balıkesir	Bursa
Virus	Leek	27/62	10/32	11/48	10/62	3/32	5/48	9/62	6/32	6/48
	Onion	1/48	0/33	2/116	0/48	0/33	0/116	0/48	0/33	0/116
	Garlic	0/40	1/98	0/17	26/40	41/98	10/17	3/40	8/98	1/17
Total		28/150	11/163	13/181	36/150	44/163	15/181	12/150	14/163	7/181
		52/494			95/494			33/494		
		180/494								

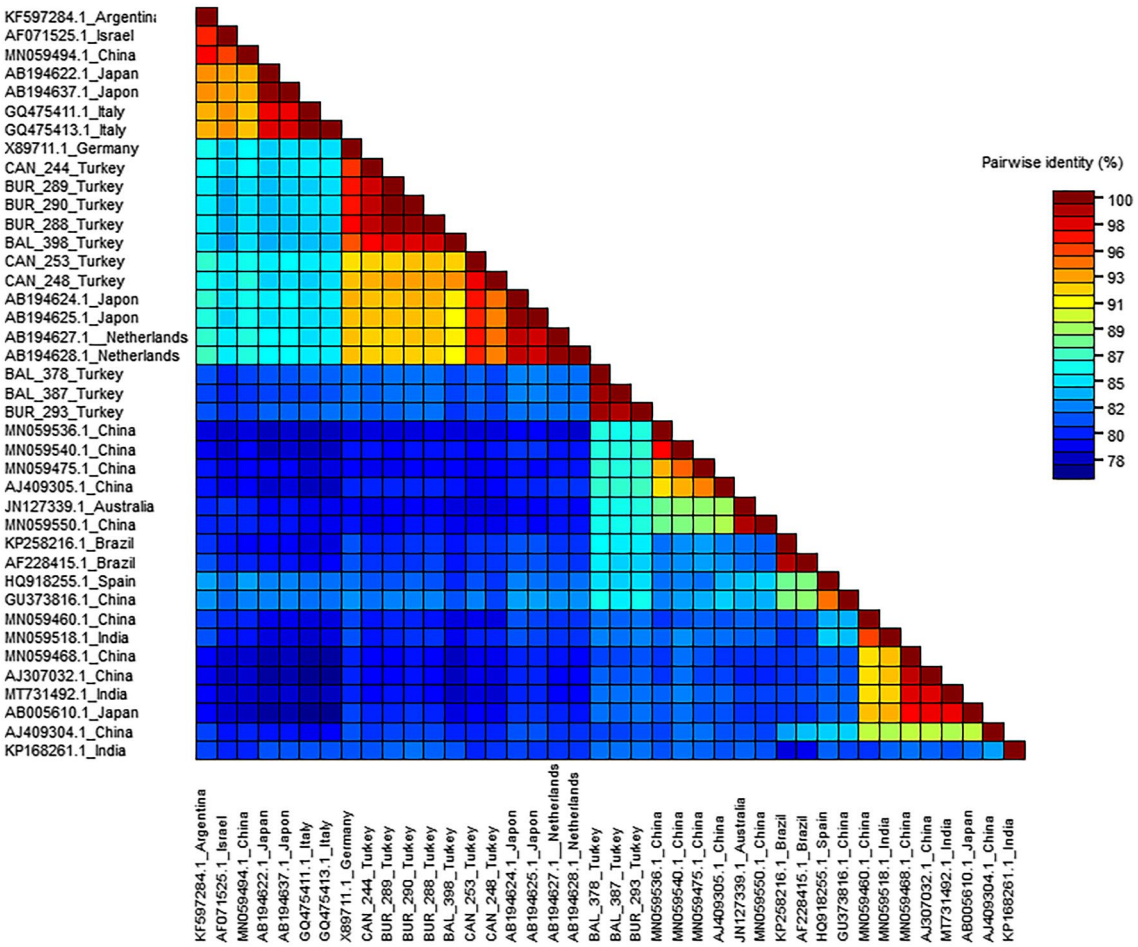

Fig. 2 The distribution of the pairwise identity scores of the complete coat protein genes of leek yellow stripe virus isolates at nucleotide level as calculated by the SDT software

As a result of the phylogenetic analysis, the LYSV isolates were divided into two major groups. All members of the first group consisted of LYSV isolates obtained from garlic as hosts that were then distributed into two subgroups, whereas the second group was divided into two subgroups consisting of garlic and leeks (Fig. 4). In the second group, the isolates obtained from garlic and leeks were distributed in different subgroups. Isolates obtained from this study were observed in the phylogenetic tree to be in close relationships with each other based on their hosts of origin.

Phylogenetic analysis of the OYDV isolates revealed that the isolates were distributed in the phylogenetic tree in two major groups. Furthermore, five subgroups were identified in the phylogenetic tree (Fig. 5). In the subgroups of the first group, the isolates were found to be distributed according to their host of origin (onion and garlic), whereas in the second group, isolates with a different host of origin (onion, garlic, and leek) were found to be in the same subgroups. It was determined that the isolates from Turkey were distributed into two different main groups (I and II) and two subgroups (Ib and IIb).

The genetic distances of all isolates used in the study were 0.1962 ± 0.0252 and 0.2476 ± 0.0394 for the LYSV and OYDV isolates, respectively (Supp. Table 4 and 5). The genetic distance of the LYSV isolates obtained from leeks was 0.0558 ± 0.0084, while it was 0.1986 ± 0.0262 for those obtained from garlic (Supp. Table 4). The genetic distances of OYDV isolates obtained from garlic, onion, and leek hosts were 0.2205 ± 0.0352, 0.2457 ± 0.0397, and 0.1704 ± 0.0270, respectively (Supp. Table 5). These calculations were also performed at the group and subgroup levels formed in the phylogenetic trees for the LYSV and OYDV isolates (Supp. Table 4 and 5).

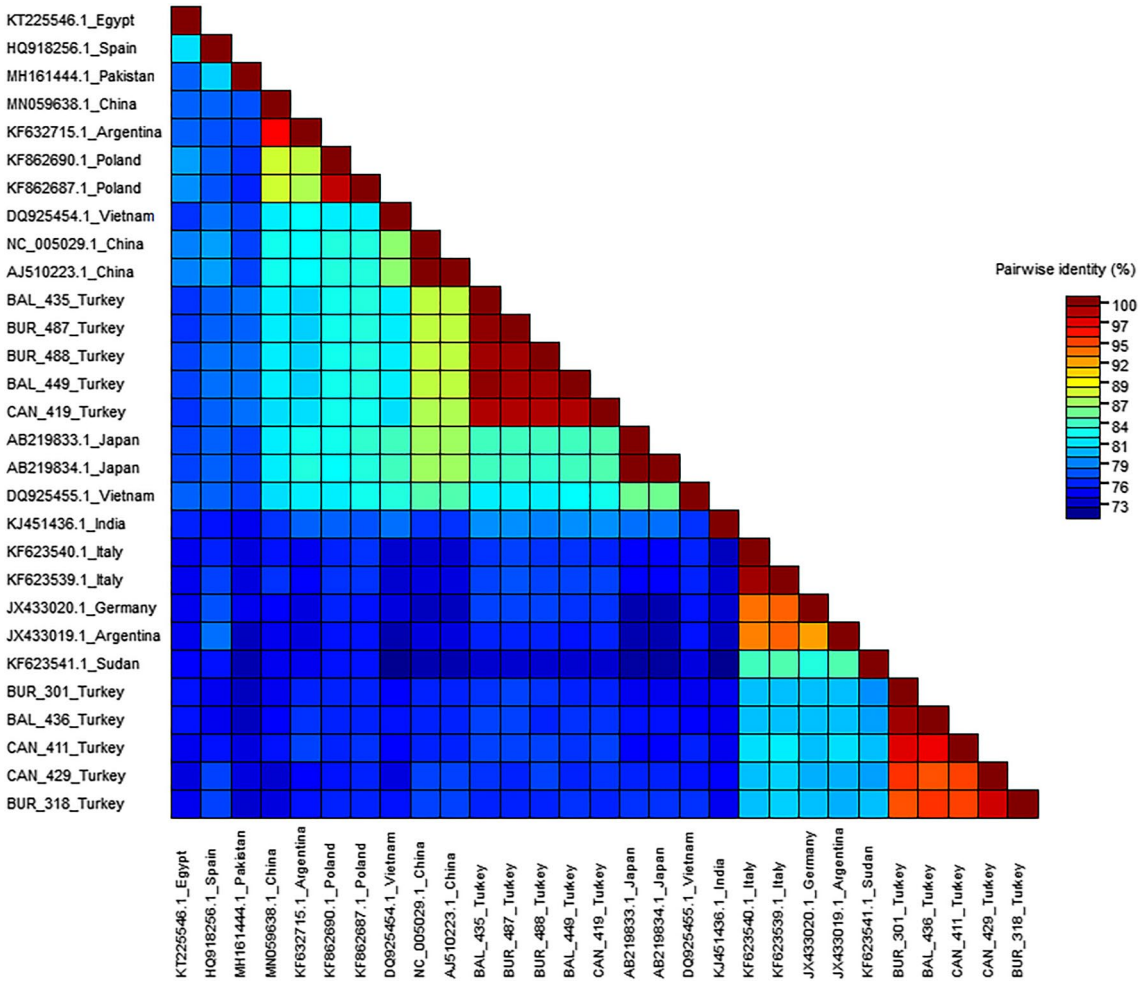

Fig. 3 The distribution of the pairwise identity scores of the partial coat protein genes of onion yellow dwarf virus isolates at nucleotide level as calculated by the SDT software

Discussion

Virus identification studies performed within the scope of this study found the virus infection rate in the collected samples was 36.43%. Although all samples typically exhibited virus and virus-like symptoms, the resulting infection rate was relatively low. It is thought that two-thirds of the collected samples were not infected with the viruses in question and were instead infected with other viral diseases that affect *Allium* plants. Furthermore, Alliaceae plants are frequently reported to be infected with carlavirus, allexivirus, and phytoplasma infections in addition to potyvirus infections (Wylie et al. 2012; Abraham et al. 2019). In particular, the fact that the virus entities in question cannot be identified in any of the collected onion samples supports the idea that the observed symptoms may be caused by infections of other viruses and virus-like diseases (Baghalian et al. 2010; Gupta et al. 2017).

In the present study, LYSV and OYDV were found to extensively infect leeks and garlic, while LYSV infection was identified in only two onion plants. Based on the data in the literature, the infection rate of OYDV in onions is low, whereas its prevalence is higher in garlic (Shahraeen et al. 2008; Snihur et al. 2019). Therefore, garlic is considered the main host of OYDV in many countries. In fact, the host of origin of most of the OYDV isolates available in the GenBank database is garlic. Thus, the results obtained in this current study are compatible with previous studies.

Although OYDV infection based on DAS-ELISA test results was detected in seven onion plants, it is believed that the test may have cross-reacted with another potyvirus because the RT-PCR tests did not confirm this finding. According to Dovas et al. (2002), the RT-PCR test is reported to be more sensitive than the DAS-ELISA test for identifying LYSV and OYDV.

The sequence diversity was observed to be high among the LYSV and OYDV isolates. Furthermore, parallel to

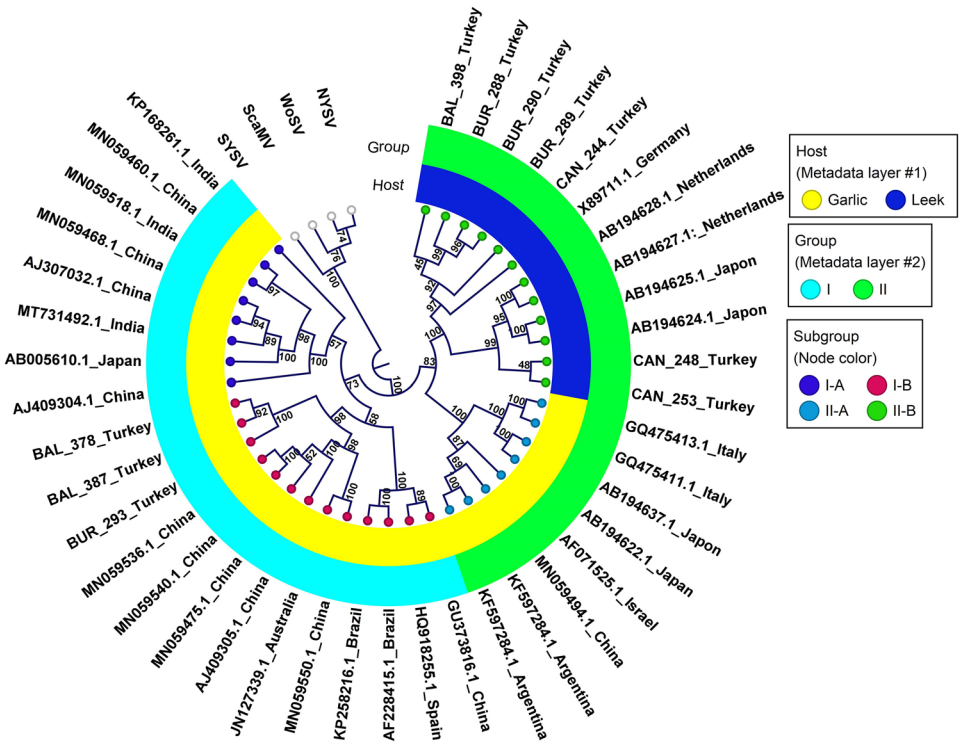

Fig. 4 The phylogenetic tree of leek yellow stripe virus (LYSV) isolates constructed by the Maximum likelihood method using the nucleotide sequences of the complete coat protein genes. Scallion mosaic virus (ScaMV, AJ310208), wild onion symptomless virus (WoSV) (LC159495), narcissus yellow stripe virus (NYSV, KU516386), and shallot yellow stripe virus (SYSV, AM267479) related to LYSV were used as outgroup for construction of rooted phylogenetic tree

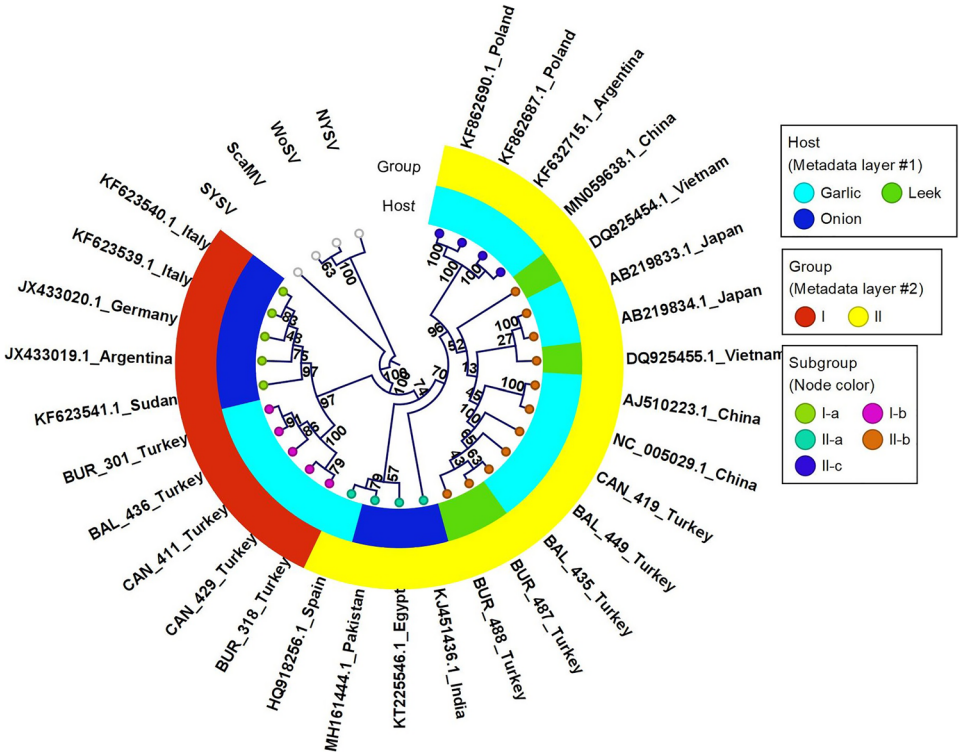

Fig. 5 The phylogenetic tree of onion yellow dwarf virus (OYDV) isolates constructed by the Maximum likelihood method using the nucleotide sequences of the partial coat protein genes. Scallion mosaic virus (ScaMV, AJ310208), wild onion symptomless virus (WoSV) (LC159495), narcissus yellow stripe virus (NYSV, KU516386), and shallot yellow stripe virus (SYSV, AM267479) related to OYDV were used as outgroup for construction of rooted phylogenetic tree

previous studies, we found that isolates obtained from different continents showed higher sequence similarity with each other, while isolates obtained from the same region exhibited very low sequence similarities (Arya et al. 2006; Sivaprasad et al. 2017). The similarity rates of LYSV and OYDV isolates among themselves and with world isolates have been observed to be very close to the threshold values required to be considered a new virus in potyvirus taxonomy

(Adams et al. 2005). Low similarity rates obtained for LYSV and OYDV isolates were also obtained in studies on OYDV from Iran and LYSV from India (Baghalian et al. 2010; Gupta et al. 2017). In this context, findings reported from other studies support the results of the present study (Takaki et al. 2005).

LYSV and OYDV isolates are reported to be phylogenetically distributed into two major groups (Chen et al. 2001; Baghalian et al. 2010; Gupta et al. 2017). Phylogenetic analyses conducted within the scope of this study also revealed that the LYSV and OYDV isolates formed two major groups. Moreover, intergroup genetic distance values between the major groups were found to be significantly greater than the intragroup genetic distance values. Similar results were also obtained for the subgroups. These findings confirm the accuracy of the groups formed in the phylogenetic tree in parallel with data in the literature. LSYV leek isolates were found to be much more closely related to each other than LYSV garlic isolates based on genetic distance measurements at the host level. Garlic LSYV isolates were found in different major groups and subgroups in phylogenetic analyses. Host specialization of OYDV isolates was found to be less common than that of LYSV isolates, and isolates with a different host of origin in different subgroups were found.

Furthermore, the bioinformatics analyses revealed that the host plays an important role in the formation of major groups in phylogenetic relationships among LYSV isolates, whereas geographic origin does not play a role in the formation of major groups in phylogenetic relationships based on the gene regions used for both LYSV and OYDV isolates. In this context, it has been determined that isolates obtained from various continents, countries, and regions can be found in the same groups. Similar results have been reported in many studies on LYSV and OYDV isolates (Bereda et al. 2015; Gupta et al. 2017).

Conclusion

We believe it is necessary to conduct studies to investigate why LYSV and OYDV do not prefer onions, although they are cultivated in a manner similar to garlic (vegetative propagation material), and the host resistance and sources of infections for these viruses are the same. Moreover, high heterogeneity among both OYDV and LYSV isolates in terms of similarity exists; therefore, it is important to carry out additional bioinformatics analyses at the level of the complete genome with isolates obtained from different geographic origins and hosts for both viruses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13205-021-03067-1.

Author contributions SK: designed the study. HTT and AK: collected the samples and performed the laboratory tests. AK and SK: performed the bioinformatic analyses. All authors interpreted of results, drafted manuscript preparation, and approved the final version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appearedto influence the work reported in this paper.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participantsincluded in the study.

References

Abraham AD, Kidanemariam DB, Holton TA (2019) Molecular identification, incidence and phylogenetic analysis of seven viruses infecting garlic in Ethiopia. Eur J Plant Pathol 155:181–191

Adams MJ, Antoniw JF, Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family Potyviridae. Arch Virol 150:459–479

Arya M, Baranwal VK, Ahlawat YS, Singh L (2006) RT-PCR Detection and molecular characterization of onion yellow dwarf virus associated with garlic and onion. Curr Sci 91:1230–1234

Baghalian K, Kim OK, Natzuaki KT (2010) Molecular variability and genetic structure of the population of onion yellow dwarf virus infecting garlic in Iran. Virus Genes 41:282–291

Bereda M, Elżbieta P, Kalinowska M, Stefan S (2015) Genetic diversity and evidence of recombination in the coat protein gene of onion yellow dwarf virus. Eur J Plant Pathol 142:377–387

Chen J, Adams MJ (2001) Molecular characterisation of a complex mixture of viruses in garlic with mosaic symptoms in China. Arch Virol 146:841–1853

Clark MF, Adams AN (1977) Characteristic of the microplate method of enzyme linked ımmune sorbent assay for the detection of plant viruses. J Gen Virol 34:475–483

Dovas CI, Hatziloucas E, Salomon R, Barg E, Shiboleth Y, Katis NI (2001) Incidence of viruses infecting *Allium* spp. in Greece. Eur J Plant Pathol 107:677–684

Dovas CI, Hatziloukas E, Salomon R, Barg E, Shiboleth Y, Katis NI (2002) Comparisons of methods for virus detection in *Allium* spp. J Phytopathol 149:11–12

Fajardo TVM, Nishijima M, Buso JA, Torres AC, Avila AC, Resende RO (2001) Garlic viral complex: identification of potyviruses and carlavirus in central Brazil. Fitopatol Bras 26:619–626

Fernández-Tabanera E, Fraile A, Lunello P, García-Arenal F, Ayllón MA (2018) First report of onion yellow dwarf virus in leek (*Allium ampeloprasum* var. *porrum*) in Spain. Plant Dis 102:256

Fidan H, Baloglu S (2009) First report of onion yellow dwarf virus and Leek yellow stripe virus in garlic in Turkey. Plant Dis 93:672

Gupta N, Islam S, Sharma SK, Baranwal VK (2017) Complete genome sequence of an isolate of leek yellow stripe virus from garlic in India. J Plant Pathol 99:793–797

Katis NI, Maliogka VI, Dovas CI (2012) Viruses of the genus *Allium* in the mediterranean region. Adv Virus Res 84:163–208

Korkmaz S, Cevik B (2009) Leek yellow stripe virus newly reported in Turkey. Plant Pathol 58:787

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Lee JB, Kim SM, Lee JS, Park JW, Choi HS, Lee JH, Lee KW, Lee SH (2005) Incidence and variation of the viruses in the northern ecotype garlic. J Virol Methods 124:55–56

Li QQ, Zhou SD, He XJ, Yu Y, Zhang YC, Wei XQ (2010) Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. Ann Bot 106:709–733

Lot H, Chevelon V, Souche S, Dellecolle B (1998) Effects of onion yellow dwarf virus and leek yellow stripe virus on symptomatology and yield loss of three french garlic cultivars. Plant Dis 82:1381–1385

Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS ONE 9:e108277

Rahman K, Lowe GM (2006) Garlic and cardiovascular disease: a critical review. Nutr J 136:736–740

Santosa AI, Ertunc F (2020) Identification, molecular detection and phylogenetic analysis of four viruses infecting *Allium cepa* in Ankara Province, Turkey. J Plant Dis Prot 127:561–569

Shahraeen N, Lesemann DE, Ghotbi T (2008) Survey for viruses infecting onion, garlic and leek crops in Iran. Bull OEPP 38:131–135

Snihur H, Shevchenko T, Sherevera K, Budzanivska I, Shevchenko O (2019) First report of onion yellow dwarf virus in Ukraine. J Plant Pathol 101:1283–1283

Taglienti A, Tiberini A, Manglli A, Rea R, Paoletti S, Taviani P, Tomassoli L (2018) Molecular identification of allexiviruses in a complex mixture of garlic viruses in Latium (central Italy). Eur J Plant Pathol 150:797–801

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. Arch Virol 150:1135–1149

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 Sci Pol Hortorum Cultus 13:123–133

Wylie SJ, Li H, Jones MG (2012) Phylogenetic analysis of allexiviruses identified on garlic from Australia. Australas Plant Dis Notes 7:23–27