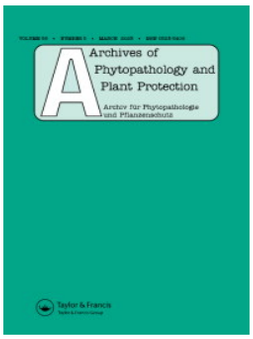

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RESEARCH ARTICLE

Status of virus and phytoplasma diseases in carrot growing areas of Ankara and Konya provinces, Turkey

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ABSTRACT

In this study, infections that may emerge from viruses and phytoplasmas were investigated in carrot cultivation fields in the Ankara and Konya provinces, which are located in the Central Anatolia Region of Turkey and have the largest agricultural areas of the country. A total of 272 symptomatic and asymptomatic carrot plants, together with their root and leaf parts, were collected from 61 fields by performing periodic field studies during the plant vegetation periods between 2018–2019. Samples were tested against carrot virus Y (CarVY), carrot red leaf virus (CtRLV), carrot red leaf virus-associated RNA (CtRLVaRNA), carrot mottle virus (CMoV), carrot torradovirus 1 (CaTV), carrot yellow leaf virus (CYLV), carrot thin leaf virus (CTLV) and cucumber mosaic virus (CMV) agents by Reverse Transcriptase-PCR (RT-PCR). To determine phytoplasmas, samples were tested using universal primers by nested PCR. Molecular tests conducted to investigate the aforementioned viruses produced negative results, but phytoplasma infections were widely found. 16SrVI-A "*Candidatus* Phytoplasma trifolii" and 16SrI-B "Aster yellows" subgroups were detected by *in vitro/in silico* RFLP and sequencing analyses. Disease incidence caused by both phytoplasma groups was 39.34%. This study determined for the first time that 16SrVI and 16SrI groups cause severe infections in the carrot fields of Turkey.

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KEYWORDS
Ankara; Konya; carrot; yellowing; redding; virus; phytoplasma; PCR

Introduction

The carrot (*Daucus carota* L.) is one of the most important species of members of a very large cosmopolitan family, the Apiaceae. Carrot plants were first cultivated on the Iranian plateau about five thousand years

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ago for their nutritious roots (Brothwell and Brothwell 1969) and are grown extensively in many parts of the world, still retaining their nutritional importance today. Ankara and Konya provinces, located in the central Anatolian region of Turkey, have the country's largest agricultural areas and are also centers for carrot production. These two regions provide 85% of the 589.000 tons of carrots produced in the country each year (TSI 2022). These outputs provide a large portion of the supply for both domestic and international market demands. Thus, this income makes a significant contribution to the economy of the region.

A variety of diseases reduce the yield and market value of carrots wherever they are grown (Davis and Nuñez 2007). Phytopathogens such as spiroplasmas, viruses, phytoplasmas, and *Candidatus Liberibacter solanacearum* have been documented to cause serious damage and economic yield losses among these diseases (Latham and Jones 2004; Cebrián et al. 2010; Clements et al. 2021). Single-infection and co-infections caused by these pathogens alone and/or in combination with more than one pathogen have been reported under field conditions (Valiunas et al. 2001; Lee et al. 2006; Gamarra et al. 2011; Alfaro-Fernandez et al. 2012). Infections by viruses and phytoplasmas can cause significant losses where carrots are grown year-round or harbour pathogen vectors and alternative hosts are available (Davis and Nuñez 2007).

At least 30 virus agents have been identified, which may or may not cause significant damage to the carrot plant, both economically and in terms of product quality (Brunt et al. 1996). Among these viral agents, carrot thin leaf virus (Family Potyviridae; CTLV), a typical potyvirus with a (+) ssRNA genome that is easily transmitted by aphid species like Myzus persicae and Caveriella. aegopodii, was first detected by Howell and Mink (1976) in Washington State carrot cultivation areas. Carrot virus Y (CarVY), another potyvirus member that causes infections in carrots, was reported for the first time from Australia and is a viral agent transmitted by aphids in its (+) ssRNA genome feature (Latham and Jones 2002). Carrot torrado virus 1 (Family *Secoviridae*; CaTV1) was first discovered in England in 2013 in the search for viruses that cause internal necrosis in carrots (Adams et al. 2014), and was later detected in Greece, Japan, and France, and is a torradovirus transmitted by aphids with the (+) ssRNA genome feature (Rozado-Aguirre et al. 2017; Lotos et al. 2018; Tokuda et al. 2019). Carrot yellow leaf virus (Fam: *Closteroviridae*; CYLV) was first detected in plants showing yellowing of carrot leaves by Yamashita et al. (1976) in Japan and by Van Dijk and Boss (1996) in Europe. CYLV is a member of closterovirus with a (+) ssRNA genome separated from ampeloviruses and criniviruses genuses because it is typically transmitted by aphids (Menzel et al. 2009). Cucumber mosaic virus (Family Bromoviridae; CMV), which is a

member of the cucumovirus type species with (+) ssRNA genome, was first reported from the Chiba and Hiratsu regions of Japan due to the mosaic symptoms it causes in carrots (Iwaki and Komuro 1970). Carrot motley dwarf complex disease (CMD) is an important disease triggered by three viral agents together in the *Apiaceae* family members. One of these agents is the carrot mottle virus (Family *Tombusviridae*; CMoV), an umbravirus with a mechanically and aphid-transmitted (+) ssRNA genome (Watson et al. 1964). The other two agents are the poleroviruses carrot red leaf virus (Family *Luteoviridae*; CtRLV) and carrot red leaf virus-associated RNA (Grouped under Virus-dependent nucleic acids: Polerovirus- associated RNAs: CtRLVaRNA), which are carried by aphids and not mechanically transmitted (+) ssRNA genome feature (King et al. 2012).

Phytoplasmas were first named as viruses by Kunkel (1926), especially the "aster yellows" disease. Later, phytoplasmas were reported as mycoplasma-like organisms (Doi et al. 1967). They are defined as bacteria that are widespread in almost all regions of the world today, infect more than a thousand plant species, are limited to phloem tissues, lack cell walls, and are transmitted by insect vectors (Harrison et al. 2014). Diagnosis and phylogenetics of phytoplasmas have historically been based on the 16S rRNA (ribosomal RNA) gene and the 16S-23S rRNA spacer region due to the availability of universal primers. However, it is well known that phylogenetics based on a single highly conserved gene has limitations, especially when it comes to describing its differences within subgroups and different strains, and the approach currently used for most other organisms is to reconstruct trees by combining sequence data from different genes (Hodgetts and Dickinson 2010). Furthermore, RFLP (restriction fragment length polymorphism) analyses of fragments obtained by amplification of a specific part of the highly conserved region of 16S rDNA (ribosomal DNA) are widely and extensively used for phytoplasma classification (Zhao and Davis 2016).

Carrot cultivation has been done in the Central Anatolian Region of Turkey for many years, particularly in the provinces of Konya and Ankara, which have the country's largest agricultural areas. The symptoms of severe reddening, yellowing, and root deformations of plants grown in these areas for a long time have been observed to have a negative impact on the yield and quality of the product. However, studies on the causes of these infections have been very limited. The presence of potyviruses was reported in a study conducted in Ankara based on serological and mechanical inoculation methods (Şakar-Çabuk 2014). In addition, in a study conducted in the Hatay province, stolbur infection was reported in carrots (Sertkaya 2014). Therefore, for the first time in Turkey, the presence of viral agents and phytoplasmas that

could cause these product losses was thoroughly investigated in this study.

Materials and methods

Field surveys and carrot sample collection

In Ankara and Konya provinces, where the continental climate is effective, samples were gathered by conducting symptomatologic observations between the spring and autumn seasons, which are near to harvest time and/or vegetative periods of the plants (Figure 1). Thus, 272 carrot samples, together with their leaves and roots, were collected from a total of 61 different fields, symptomatic and/or non-symptomatic, by conducting periodic field surveys between 2018 and 2019, April–September. Once the symptomatic information was recorded, the root and leaflets of carrot samples were stored in a deep freezer at −20 °C for use in molecular experiments.

Total nucleic acid (TNA) isolation and molecular assays

Total nucleic acid (TNA) isolations were performed initially to molecularly determine the presence of viruses and phytoplasmas in carrots. With minor modifications, the isolation procedure was carried out using 100 mg of leaf tissue, as Li et al. (2008) described. The quality and quantity of RNA and DNA extracted from the samples were spectrophotometrically assessed using a Nano-drop (Thermo Scientific, USA) and stored at −80 °C for use in molecular synthesis and amplifications.

Figure 1. Representation of the research regions on the country map (Ankara and Konya/Turkey).

Since all of the viruses in the research have RNA genome features, cDNA synthesis has been carried out primarily to use in amplification. In a total volume of 20 µl for each sample, the synthesis reaction contained about 1000 ng/µl RNA, 0.2 µg/µl Random Hexamer primer (5′-NNNNNN-3′), 100 units of Reverse-Transcriptase enzyme (200 U/1 µl) (Thermo Sci. 200 U/1 µl), 10 units of RNasin (RNAse inhibitor; Thermo Sci., 40 U/1 µl), 1.5 µl (10 mM) dNTP, 1X RT-buffer (250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT), and DEPC (diethylpyrocarbonate) -water. Tubes were incubated in a thermal cycler (Biometra, Germany) for 3 min (denaturation) at 95 °C, 15 min at 25 °C (primer annealing), and 60 min at 42 °C (enzyme formation activity). The synthesis reagents were obtained and then stored at −20 °C for use in amplification processes. The cDNAs obtained by synthesis reactions were used in polymerase chain reactions with specific primers listed in Table 1 at the appropriate conditions and temperatures. Thus, CMoV, CtRLV, CtRLVaRNA, CaTV1, CYLV, CTLV, CMV-specific primers, and degenerate potyvirus primers were used to detect these RNA viruses.

Table 1. Primer pairs used in the molecular assays of viruses that cause infections in carrots.

Viral agent	Primer sequences (5′ - 3′)	Expected fragment size	Target region and references
CTLV	F-ACCAAGGAATGGAACGGGAGGG	1192 bp	NIb ve CP
	R-TCAAGACAAACCCATTACATAGTACG		Xu et al. (2014)
CTLV	F-GAAGTTCGGATGCTAGATGAGTTAC	753 bp	Polyprotein
	R-GTTGAATTGGTTGTGTGTTGATCTTG		Lotos et al. (2018)
CtRLV	F-GAGGTGAGAAATCGCYTGAC	211 bp	RdRp
	R-MGGCGCCACARTGATAGG		Vercruysse et al. (2000)
CtRLV	F-GAGAGCAACAGGAATTAAAC	436 bp	CP
	R-TTTGTAGATTGTGCTCGAAAGTT		Oka et al. (2012)
CtRLV	F-CACACCTTCAAGGCGCCCACG	336 bp	CP
	R-ATCGAGCCACCGGCGGTTGA		Oka et al. (2012)
CMoV	F-TGGWGTICACAACAACTC	408 bp	RdRp
	R-AAGGCTTTGTACAACATTGG		Vercruysse et al. (2000)
CMoV	F-CAACTCCCTCAAGAACCTCGT	321 bp	RdRp
	R-GGTCAGGTTTGGCTGTGAAG		Naseem et al. (2016)
CtRVaRNA	F-TCTAGTTTCTCTCAAGTTCCA	486 bp	RdRp
	R-CCTCACCTRCCAATTATGG		Vercruysse et al. (2000)
CtRVaRNA	F-CTCTCAAGTTCCAGTACTTGG	309 bp	RdRP
	R-TGGACATGCTCAATTGGTG		Naseem et al. (2016)
CtRVaRNA	F- TCTAGTTTCTCTCAAGTTCCA	737 bp	RdRp
	R- ACCCTCCCCTGTAGTTC		Vercruysse et al. (2000)
CaTV1	F-TCAATCAGTATTAAGCGAGGAATGG	262bp	Polyprotein gene
	R-CCTCAATGGGCTTGTAATGA		Rozado-Aguirrea et al. (2016)
CaTV1	F-CTTTCCACCAATATTATGCAAGTTCC	670 bp	RNA2
	R-ATAAAAAATCCACTCCGCTTCTTCC		Lotos et al. (2018)
CMV	F-YASYTTTDRGGTTCAATTCC	940 bp	RNA3
	R-GACTGACCATTTTAGCCG		Choi et al. (1999)
CYLV	F-CGGTTTGGTGAGTTTGT	813 bp	CP
	R-ATGGCTGGAGGTTAGGA		In this study
CarVY	F-GTITGYGTIGAYGAYTTYAAYAA	350 bp	NIb
	R-TCIACIACIGTIGAIGGYTGNCC		Zheng et al. (2008)
CMV	F-GTTTATTTACAAGAGCGTACGG	650 bp	2a protein
	R-GGTTCGAA(AG)(AG)(AT)ATAACCGGG		Sialer et al. (1999)

In addition, CTLV, CMoV, CtRLV, CMV, and CarVY positive control samples were provided by other researchers and used in the investigation of viruses at the molecular level.

Nested-PCR studies were carried out using TNAs to determine the presence of phytoplasmas in carrot plants. TNAs were diluted with DNA concentrations of 20–40 ng/µl per reaction, and Nano-drop 2000 (Thermo Sci., USA) was used for nucleic acid measurements. The total reaction volume is 25 µl; the first step with 1 µl (20–40 ng/µl) gDNA, 1.8 mM $MgCl_2$, 0.4 µM P1/P7 (Deng and Hiruki 1991), and/or R16mF2/R16mR2 (Gundersen and Lee 1996) primer pairs, 1X PCR buffer, 0.4 mM dNTP, and 1.25 units of Taq polymerase (5 u/1 µl) (Ampliqon, Denmark) was performed. The reactive products obtained from this first amplification were diluted with nuclease-free water at 1:30 ratios and used as templates in the second reaction. In the amplification, the other universal primers R16F2n/R16R2 (Lee et al. 1993), fU5/rU3 (Lorenz et al. 1995), and M1 (758 F)/M2 (1232 R) (Gibb et al. 1995) pairs were used. The PCR products were run in a 1X TAE buffer solution on a 1% agarose gel at 100 V for 1 h. Agarose gels were treated with ethidium bromide and then checked with a UV imaging device (Gene genius, UK).

In-vitro and in-silico PCR-RFLP analysis of phytoplasmas

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analyzes approximately 1.25 kb amplicons obtained from nested-PCR assays using R16F2n/R16R2 primers, which were performed with *TaqI* and *AluI* endonuclease enzymes *in vitro* conditions. This analysis was performed at 65 °C for the *TaqI* enzyme and at 37 °C for the *AluI* enzyme in accordance with the protocol recommended by the manufacturer (Eurx, Estonia). Also, the restriction DNA patterns of infected carrot samples were compared with the reference control (Cabbage chloranthy; CHLL, *Catharanthus roseus,* and Brinjal Little Leaf, BLL) RFLP patterns. In addition, samples representing the digestion profiles obtained under *in vitro* conditions and the regions where the infection was detected were selected, and after their sequence analysis was completed, computer-simulated *in silico* PCR-RFLP digestions were made using the *iPhyclassifier* software (Zhao et al. 2009).

Sequencing and molecular evaluation analysis

According to *in-vitro* PCR-RFLP patterns, samples were chosen to represent each surveyed region and bi-directionally sequenced to reveal nucleotide sequences. Using NCBI (National Center for Biotechnology Information) and *iPhyclassifier* software, the nt homology ratios of the

samples whose nt sequences were determined were calculated. Also, twenty-one carrot phytoplasma isolates were uploaded to GenBank under the accession numbers MZ452938-MZ452950 (16SrVI) and MZ457911-MZ457918 (16SrI). To begin using phylogenetic analysis, isolates from different parts of the world were taken from the GenBank and aligned with Turkish isolates using ClustalW (Thompson et al. 1994). For the analysis of aligned data, the tests were continued by using the Best Find model in the MEGA X v.10.2.4 software (Kumar et al. 2018), considering the lowest BIC (Bayesian Information Criterion) value. A phylogenetic tree was constructed with a 1000-bootstrap, statistical analyses were carried out using the Maximum-Likelihood (ML) method, and the substitution model was applied using the Tamura-3 parameter (T92 + G) gamma distribution in the MEGA X. Furthermore, *Acholeplasma laidlawii* (acces no M23932), a non-cell wall bacteria genetically related to phytoplasma, was used as an out-group to increase the reliability values of the dendrogram's main branches.

The mean evolutionary distance of phytoplasma isolates in the 16S rRNA gene within and between ribosomal groups (AY) 16SrI, 16SrXII, and 16SrVI main groups were determined by calculating using the Tamura-3 parameter (T92 + G) in MEGA X v.10.2.4, with estimates of standard error (SE) obtained using a bootstrap procedure (1000 replicates).

Results

Field surveys

As a result of the field surveys that continued for two years, a total of 272 carrot samples were collected from 29 different fields in two regions (Beypazarı and Nallıhan) of Ankara and 32 different fields in four regions (Çumra, Meram, Karapınar, Ereğli) of Konya. Severe, mild, and moderately varying reddening/purpling, yellowing, chlorosis, weak foliation, and rarely necrotic symptoms were observed in the leaf parts of the plants (Figure 2). In the root parts, symptoms such as growth retardation, lateral root formation, bubbling on the root surface, elastic root structure, and necrotic tissues were recorded (Figure 3).

Amplification results of viruses and phytoplasmas

Fragments of expected sizes were obtained from CMoV, CTLV, CMV, CtRLV, and CarVY positive control samples when viruses were examined using conventional RT-PCR methods. However, no amplicons of the expected size were obtained from any of the carrot samples tested in this study, and all results were negative.

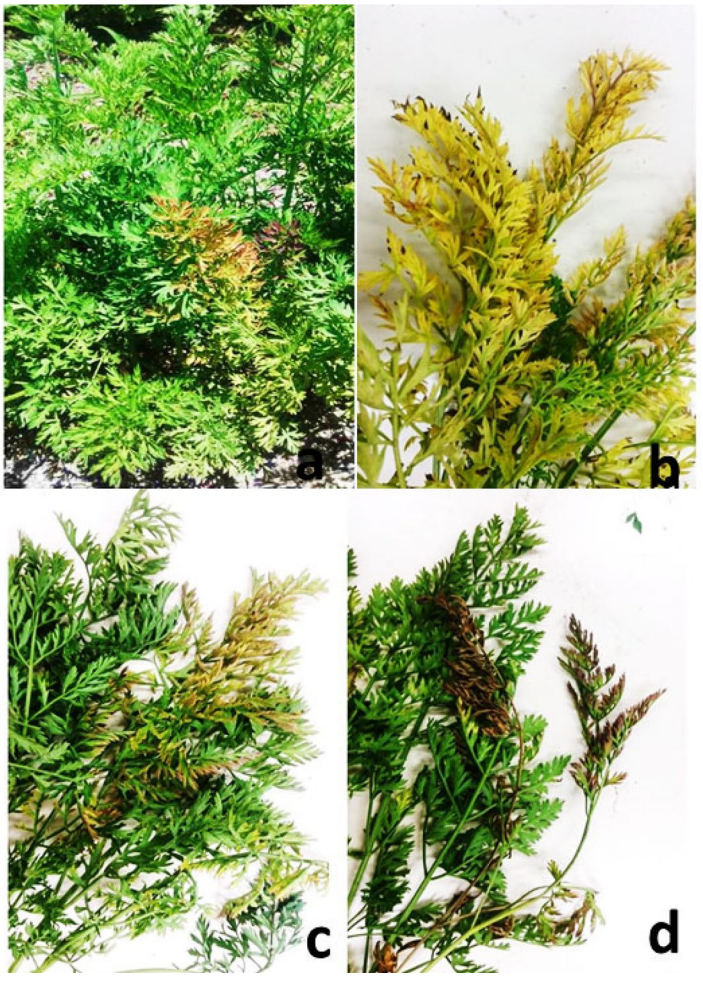

Figure 2. Symptoms exhibited by infected carrot plants (a and b) varying reddening/purpling and yellowing symptoms on the leaf parts of the plants (c and d) necrotic tissues and yellowing.

Five different universal primer sets, which are commonly used in the detection of phytoplasmas, yielded different amplification results. Amplicons of the expected size were obtained from 82 samples, 122 samples, 205 samples, 107 samples, and 193 samples using the P1/P7, R16mF2/R16mR1, F2n/R2, fU5/rU3, and M1/M2 primer sets, respectively.

RFLP analysis

The RFLP analysis results revealed two different profiles of DNA banding in each digested sample using *AluI* and *TaqI* enzymes (Figures 4 and 5). When compared to the restriction patterns of reference samples, these analyses showed that carrots were infected by two distinct groups

Figure 3. Symptoms exhibited by infected carrot roots (a) growth retardation and laterally root formation (b) bubbling on the root surface and elastic root structure (c) laterally root formation.

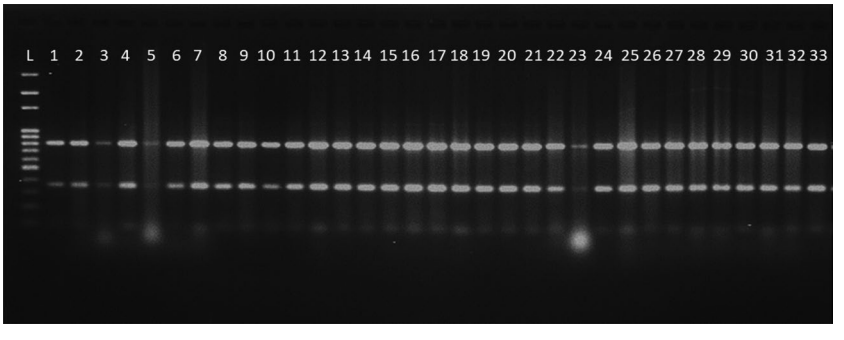

Figure 4. *TaqI* enzyme digest profiles of 1.2 kb amplicons from infected carrots (L: Ladder 100 bp) (1-30): Carrot strains (16SrVI and I), Reference strains (31-32): 16SrI-F (acces nos MZ450789 and MZ450791) (33): CHLL (16SrI-B).

of phytoplasma. Thus, these patterns obtained *in vitro* indicated the presence of *Ca.* P. asteris and *Ca.* P. trifolii strains. Furthermore, the phylogenetic analysis results were supported by collective RFLP models derived from the 16S rRNA gene fragment by *iPhyclassifier*, revealing that carrot phytoplasma isolates belonged to the 16SrVI-A and 16SrI-B subgroups (Figures 6 and 7). According to the patterns obtained from these restriction profiles, infections caused by 16SrVI-A (clover proliferation) groups and in 57 of them 16SrI-B aster yellows groups were

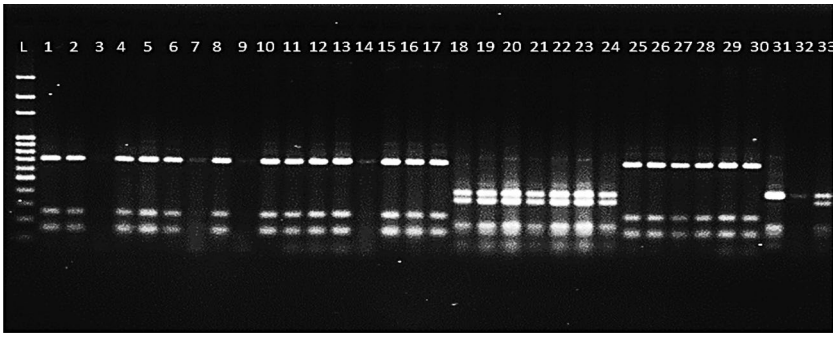

Figure 5. *AluI* enzyme digest profiles of 1.2 kb amplicons from infected carrots (L: Ladder 100 bp) (1-30): Carrot strains (16SrVI and I), Reference strains (31-32): 16SrI-F (acces nos MZ450789 and MZ450791) (33): CHLL (16SrI-B).

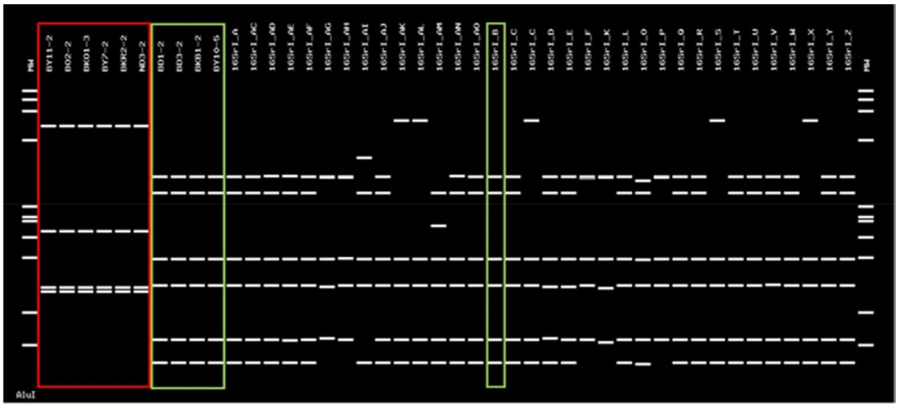

Figure 6. The digestion profiles of 16SrI and 16SrVI Turkish carrot strains formed in silico with Alu I enzyme and comparison with other 16SrI subgroups. Turkish carrot strains in the red rectangle differed from the 16SrI subgroups. Turkish carrot strains in the green rectangle formed a similar pattern with the 16SrI-B subgroup.

determined in 50 of 272 carrot samples collected from both Ankara and Konya provinces (Table 2). Infections caused by both phytoplasma groups were 39% in the carrot cultivation areas of the Ankara and Konya provinces (Table 2).

Phylogenetic inference, sequence similarity, and genetic distance

The nucleotide similarity ratios of the phytoplasma isolates obtained for this study were compared using the NCBI and the *iPhyclassifier* software. According to this, it was determined that 13 Turkish carrot isolates showed nt homogeneity between 99.5% and 99.7% with the "*Ca*. P. trifolii" 16SrVI-A subgroup isolate with accession number AY390261 in the phytoplasma software program. However, these isolates showed

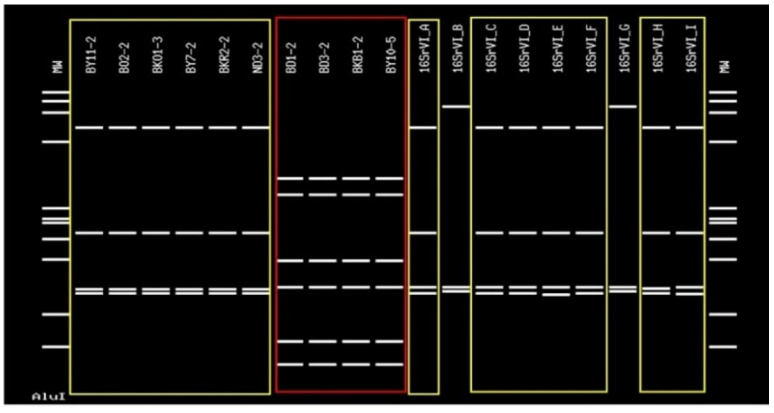

Figure 7. The digestion profiles of 16SrI and 16SrVI Turkish carrot strains formed in silico with Alu I enzyme and comparison with other 16SrVI subgroups. Turkish carrot strains in the red rectangle differed from the 16SrVI subgroups. Turkish carrot strains in the yellow rectangle formed a similar pattern with the 16SrVI-A/C/D/E/F/H/I subgroup.

Table 2. Infection distributions and incidence of phytoplasma groups according to RFLP analyzes.

Province	Location	Total number of samples / Total number of fields	*Ca.* P. asteris infection **Number samples / Number fields**	*Ca.* P. trifolii **infection Number samples / Number fields**	**Disease** Incidence **%**
Konya	Karapınar	12/4	–	6/3	50
	Ereğli	32/9	–	11/7	34.38
	Çumra	51/10	4/2	11/5	29.41
	Meram	54/9	15/8	3/1	33.33
Ankara	Beypazarı	108/24	25/10	24/12	45.37
	Nallıhan	15/5	–	8/3	53.33
Total		**272/61**	**44/20**	**63/31**	**39.34**

99.84–100% high nt homology with Chinese isolates of "Cucurbita pepo phytoplasma" (acces no KP119494) Iranian and "Brassica juncea phyllody phytoplasma" (acces no MK660149) included in the GenBank. On the other hand, the Turkish 16SrVI-A group carrot isolates showed 99.81–100% nt similarity among themselves. Moreover, eight carrot isolates showed a nt similarity of 99.5–99.8% with the "*Ca*. P. asteris" isolate with accession number M30790 in the phytoplasma software program and were matched by the software with the reference 16SrI-B isolate with accession number AP006628. Also, these isolates showed 99.44–99.92% nt homology with the "Maize bushy stunt phytoplasma" (acces no HQ530152) Colombia strain and "North American grapevine yellows phytoplasma" (acces no KX364387) the USA isolates from GenBank. Ankara and Konya isolates (16SrI-B) had 99.92–100% nt similarity among themselves.

The 16Sr I/VI/XII groups were clustered into three separate main branches in the evolutionary tree generated using the maximum

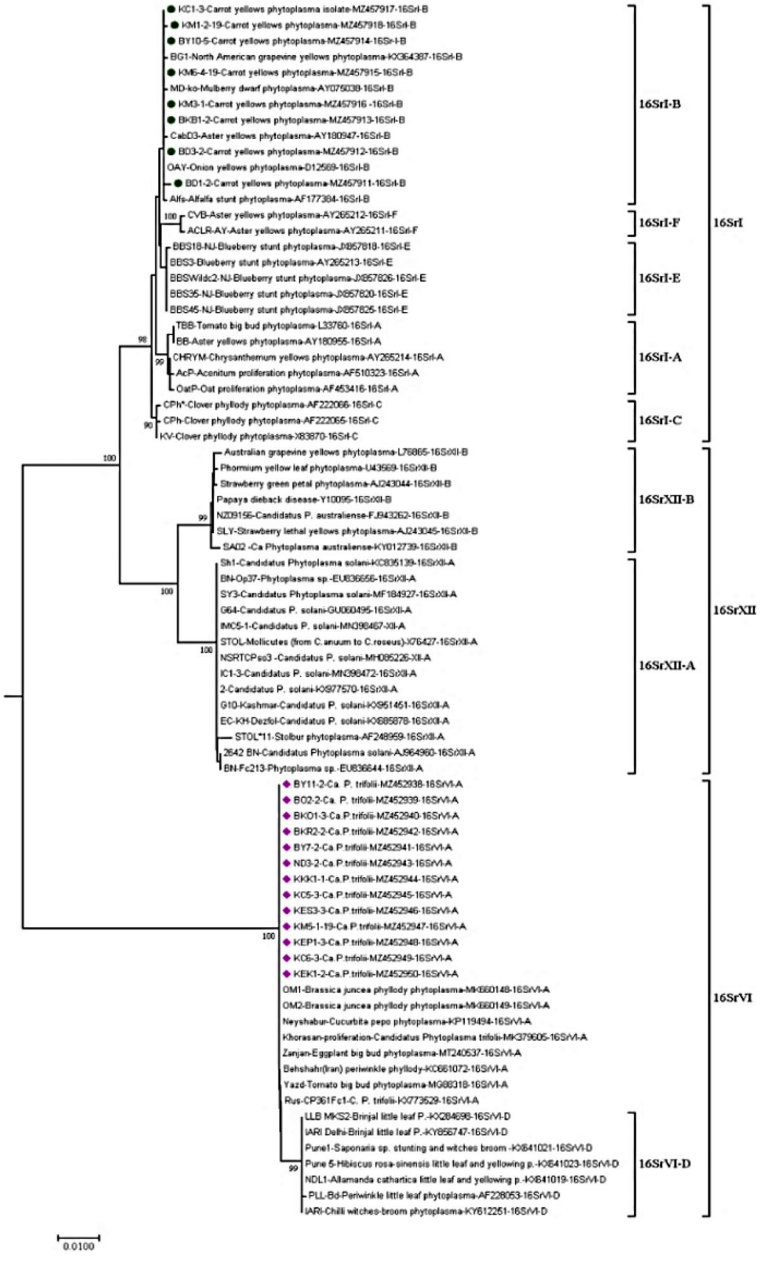

Figure 8. Phylogenetic tree generated using phytoplasma isolates from Turkish carrot strains and 16S rRNA region from other geographic strains (Isolates from this study are marked with green circle '16SrI' and pink rhombus '16SrVI').

likelihood method. Turkish carrot phytoplasma isolates were grouped with 16SrI-B and 16SrVI-A subgroup strains, and their nodes were supported with a confidence level of more than 90% (Figure 8). The genetic distance values of the isolates evaluated within their respective AY (16SrI), STOL (16SrXII), and CP (16SrVI) subgroups were all

considered to be significantly low. Furthermore, the genetic distance between subgroups STOL and CP (0.1124 ± 0.0110) was estimated to be the greatest in subgroup comparisons. However, strains of AY and STOL clustered in the same main lineage were genetically more closely related (0.0376 ± 0.0053) to each other than strains of the proliferation group-16SrVI clustered in a separate lineage. Lastly, the genetic distance between AY and CP was calculated to be 0.1003 ± 0.0102. In light of these findings, the genetic distance values obtained between the AY, STOL, and CP subgroups support the genealogical relationship between these main groups.

Discussion

Using conventional molecular techniques, this study aimed to reveal the status of viruses and phytoplasmas that cause severe redding/purpling and yellowing symptoms and thus negatively affect product yield and quality in the carrot cultivation areas of Ankara and Konya provinces, which provide economically significant income. Thus, carrot samples were collected during the vegetation and harvest periods of the plants throughout field studies conducted between the spring and autumn seasons.

First, the presence of CtRLV, CMoV, and CtRLVaRNA agents was investigated in our study based on the intense redding/purpling and yellowing symptoms seen in plants. These CMD-causing viruses have been reported in Australia, Europe, Japan, North America, and Israel (Gungoosingh-Bunwaree et al. 2009), but they have yet to be determined in this study and in our country. Furthermore, these agents have been reported to be transmitted in nature by *Cavariella aegopodii* and *Myzus persicae* aphid species in a persistent and circulative-non-propagative manner (Elnagar and Murant 1978; Vercruysse et al. 2000; Naseem et al. 2016), but these aphid populations were not found in our survey areas. However, CYLV is not a common virus in the world; it has been reported in Japan, the Netherlands, and England, sporadic infections have been observed in the areas where it has been found, and there is insufficient information about its spread (Yamashita et al. 1976; Brunt et al. 1996; Van Dijk and Boss 1996; Adams et al. 2014). Adams et al. (2014) conducted the most comprehensive study on this agent in carrot cultivation areas in England, finding that the virus causes internal necrosis of plant roots and severe yellowing of leaves. Internal necrosis was not found in carrot roots in our study, but there was a lot of yellowing in the leaves. The CYLV was unable to be identified within the scope of this research. Limited to members of the natural host line *Apiceace,* CTLV has been reported to cause discoloration of carrot leaf veins and

thinning of leaves but is not common in the world (Howell and Mink 1976; Falk et al. 1991; Xu et al. 2014).

CarVY has been associated with severe crop losses in Australian carrot plantations, with symptoms including chlorotic mottling, necrosis, and reddening of leaflets, as well as root deformations (Latham and Jones 2002; Latham et al. 2004; Jones et al. 2005). Similar leaf symptoms were observed in our field studies, but serious root deformations were not recorded, and CarVY was not detected herein. More specifically, using potyvirus universal primers, the presence of the watermelon mosaic virus (WMV) agent was identified in wild carrots exhibiting mosaic symptoms in the province of Çanakkale in Turkey's southern Marmara region, and then the agent's presence was then verified using specific primers (Randa-Zelyüt et al. 2022b). However, using the same universal potyvirus primers as in this study, no results were found in molecular studies. This situation can be explained by the climatic differences of the regions where the aforementioned survey areas are located (Marmara-temperate climate Central Anatolia- continental climate) and the high population density of a pathogen whose host is constantly present in the region. Furthermore, studies on infections caused by CMV in carrots are very limited and were first suspected in 1970 when plants from the Hiratsuka region of Japan exhibited mosaic symptoms (Iwaki and Komuro 1970). It was also reported from India by Afreen et al. (2009) in later studies that CMV caused severe chlorosis and root deformation in carrots. In this study, both symptomatologically mosaic symptoms were not recorded in the leaflets of carrot plants, and the agent was not determined molecularly.

The CaTV1 agent was first discovered by Adams et al. 2014 in a study in England, by investigating the viruses that cause internal necrosis in the roots of carrot plants with new-generation sequencing methods. After this first detection, the presence of the agent has been reported in *Torilis arvensis* ssp. *arvensis*, a weed plant in Greece, carrots in France, and *Angelica keiskei*, a medicinal aromatic plant in Japan (Rozado-Aguirre et al. 2017; Lotos et al. 2018; Tokuda et al. 2019). However, due to the co-existence with different carrot viruses (CtRLV, CMoV, etc.), the symptoms they cause in infected plants are not fully understood and the stages of symptom development are very difficult to define. In this study, however, no specific symptom that may be caused by CaTV1 was defined due to the suspicion that many different factors could be found according to the symptomatic records. In addition, positive results were not obtained from any sample in RT-PCR studies using molecular tools.

Phytoplasma infections in vegetables have been reported from five continents and forty-seven different countries of the world. In addition, among the common groups that cause these infections in vegetables,

aster yellows (16SrI) subgroups are more dominant, followed by peanut witches' broom (16SrII), clover proliferation (16SrVI), and stolbur (16SrXII-A) groups (Kumari et al. 2019). Among these vegetables, especially carrot plants, infections caused by 16SrI (A-B), 16SrII-C, and 16SrXII groups from Europe (Font et al. 1999; Duduk et al. 2008; Nisbet et al. 2014) and 16SrII (B-D), 16SrV and 16SrXII-A groups from Asia (Orenstein et al. 1999, Weintraub and Orenstein 2004; Omar 2017) have been documented. In the province of Hatay, which is located in the southern part of Turkey and has a Mediterranean climate, stolbur (16SrXII-A) infections have been reported in carrots (Sertkaya 2014). In our study, 16SrI-B 16SrVI-A subgroup phytoplasma infections were detected widely in the provinces of Ankara and Konya in the Central Anatolia Region, where the continental climate is dominant. These findings indicate that infections caused by the 16SrI-B subgroup, which are common in European carrot cultivation areas, are also present in Turkey's largest carrot cultivation areas (Table 2). Furthermore, diseases caused by the 16SrVI-A group have only been reported from Western America's carrot-growing regions, and infections caused by the same group have been identified in our study (Shaw et al. 1990; Blomquist 2002). It has been revealed through research that the 16SrVI proliferation group phytoplasma has begun to spread across our country's various geographical regions (Table 2). Particularly, 16SrVI-A group phytoplasma infections were determined in pepper, tomato, and cabbage cultivation areas (Serçe and Yılmaz 2020; Yılmaz et al. 2019, Usta and Güller 2020). Furthermore, the symptoms induced in the same host by phytoplasmas belonging to genetically different groups in vegetables can be similar or different (Martini et al. 2018). In addition, no distinction between the symptoms caused by the 16SrVI-A and 16SrI-B subgroups in the host carrot plant was observed in this study (Figure 2).

The universal primer sets P1/P7, R16mF2/R16mR1, F2n/R2, fU5/rU3, and M1/M2 used for phytoplasma diagnosis produced inconsistent PCR results in our study. Thus, it was detected that P1/P7 and R16F2n/R16R2 primers amplified both Bacillus genes and plant chloroplast rDNA at expected amplicon sizes (Harrison et al. 2002; Nejat et al. 2009). In the present study, the R16mF2/R16mR2 primers were used to eliminate false positives, and similar results were obtained. However, it was determined that the genes belonging to the *Spiroplasma* species were amplified based on the sequencing results of some fragments obtained with this primer set. In terms of ecological niches and life cycles, the genus *Spiroplasma*, which belongs to the *Mollicutes* class phylogenetically, is quite similar to *Candidatus* Phytoplasma (Gasparich 2010). As a result, some of the R16mF2/R16mR2 primers set results were not surprising. Furthermore, amplicons obtained from other M1

(16R758f)/M2 (16S1232r) primer sets yielded fragments of the expected size, but some findings indicated *Ca. Liberibacter solanacearum* organism with sequencing studies. Similar results were found by Satta et al. (2016) in the phytoplasma and liberibacter tests of the carrot plant. The fU5/rU3 primer sets produced more consistent molecular findings as a result (Ghosh et al. 2013).

In spite of the fact that insect vectors are primarily responsible for the spread of phytoplasma infections, a recent study determined that carrot seeds were contaminated with the 16SrI group (Carminati et al. 2019). In another study conducted later, it was determined that seedlings obtained from carrot seeds were infected with 16SrI and 16SrXII phytoplasma groups (Satta et al. 2020). We revealed that the 16SrVI and 16SrI phytoplasma groups were present in the seedlings in Turkey that were cultivated from seeds of various carrot cultivars that were widely grown in Ankara and Konya (Randa-Zelyüt et al. 2022a). It is abundantly clear from all of these findings that the prevalence of 16SrI and 16VI groups in the provinces of Ankara and Konya is also connected to seeds considered for this study. Controlling vegetable seeds is necessary to lower the prevalence of these groups with broad host ranges.

Conclusion

Carrot has been grown in cool and warm climate conditions for many years in our country and around the world, and although its historical background and geographical origin are not clear enough, geneticists defend the idea that Turkey and some Asian countries are the gene centers of the plant. This root vegetable has been grown for many years in our country, and it is becoming the most important agricultural economic plant in Ankara and Konya provinces. However, there was no research on viruses and phytoplasma infections that affect the quality and yield of the product and do not have direct chemical control. Based on farmer suggestions and complaints, we investigated important viral agents and phytoplasmas as research subjects in this study. While we expected viral agents to be the main causes of severe leaf redness, yellowing, and root deformation, we were surprised to find that these symptoms were caused by phytoplasmas. Foliar symptoms caused by viruses on vegetables, such as yellowing, dwarfism, and witch's broom, can be confused with symptoms caused by phytoplasmas (Kumari et al. 2019). The causes of severe infections in the carrot plant have been revealed using conventional molecular tools and methods. Although the viral agent could not be identified within the scope of this study, we do know that these pathogens cause infections in nature and have

symptoms that are similar. To provide appropriate and accurate control measures in comprehensive infection studies in Apiaceae family members, the presence of these phytopathogens should be examined together rather than focusing on a single pathogen.

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Disclosure statement

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics approval

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

Author contributions

Filiz Randa-Zelyüt and Filiz Ertunç designed the study; Filiz Randa-Zelyüt conducted the molecular experiments, and phylogenetic and PCR- RFLP analyzed the results. Filiz Randa-Zelyüt and Filiz Ertunç prepared the manuscript. Funding acquisitions were performed by Derya Şenal. All authors have read and approved the final manuscript.

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