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The association of 16SrVI and 16SrI phytoplasma groups with carrot seeds and weeds in Ankara and Konya provinces in Turkey

Türkiye'de Ankara ve Konya illerinde 16SrVI ve 16SrI fitoplazma gruplarının havuç tohumları ve yabancı otlarla ilişkisi

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A B S T R A C T

The inoculum sources of phytoplasmas associated with carrot severe yellowing and reddening symptoms were investigated in Ankara and Konya provinces, Turkey. The presence of 16SrVI and 16SrI-related phytoplasmas in the seeds of seven carrot cultivars that are widely cultivated in the regions, as well as in weeds in the fields, was determined in this study. Sequence analysis was confirmed existing phytoplasma groups in samples were determined by using conventional molecular methods (nested-PCR) and the obtained results were supported by phylogenetic studies. In addition, the obtained nucleotide sequences were compared with the reference phytoplasma sequences by in silico PCR-RFLP analysis. *Daucus carota* wild, *Medicago sativa*, *Conium maculatum*, and *Sinapis arvensis* weeds were infected with the 16SrVI (Clover proliferation) phytoplasma group. In addition, 16SrVI and 16SrI (Aster yellows) phytoplasma groups were identified in seedlings germinated from seeds of seven carrot cultivars: one was a local red carrot cultivar and six were commercially produced cultivars. To our knowledge, this was the first report of carrot seeds infected with the 16SrVI group and the presence of the 16SrVI group in *S. arvensis*, *C. maculatum*, and *D. carota* wild.

INTRODUCTION

Carrot (*Daucus carota* L.), a member of the family Apiaceae, is grown and consumed in almost every geographical region of the world. Turkey produces an average of 588.778 tons of carrot from 10.989 hectares annually, of which about 84% of production is carried out in Ankara and Konya provinces located in central Anatolia (TSI, 2020). Supply for both domestic and foreign market demands is mainly covered by these outputs. Thus, the earnings contribute significantly to the region's economy.

Several pathogens such as spiroplasmas, viruses, and *Candidatus* Liberibacter solanacearum have been reported to cause economic yield losses in carrot production areas (Cebrián et al. 2010, Latham et al. 2004, Satta et al. 2016). These pathogens have been often found together under field conditions (Alfaro-Fernandez et al. 2012, Cebrian et al. 2010, Gamarra et al. 2011, Lee et al. 2006); however, there were also reports for single-pathogen infection (Trkulja et al. 2021, Valiunas et al. 2001). In addition to carrots, up to 100% yield

losses in certain vegetables have been reported by phytoplasma-caused epidemics (Ember et al. 2011, Kumari et al. 2019).

Phytoplasmas are prokaryotic organisms without rigid cell walls and are limited to plant phloem tissues and are phylogenetically relevant to G+C Gram-positive bacteria (Weisburg et al. 1989). In general, phytoplasma infections cause some morphological symptoms on carrot plants such as reddening/yellowing of leaves, shoot proliferation, and decreased root quality (Satta et al. 2020). Other than that, phytoplasmas have been shown to also significantly impact the development of flower organs (Pracros et al. 2006).

For several decades, effective methods for identifying and characterizing phytoplasma infections on plants were not available (Kumari et al. 2019). Nowadays, different phytoplasma groups/subgroups can be characterized by utilizing molecular techniques and RFLP analysis methods based on the conserved 16S rRNA gene sequence (IRPCM 2004, Lee et al. 1995). In addition to the extensive use of conventional molecular techniques in the diagnosis of phytoplasma, quantitative-PCR techniques which produce more sensitive results, have also been applied (Liu et al. 2017). Furthermore, the high-quality availability of sequence analyses allows in silico simulations of restriction digestions and high-throughput identification in the classification of various phytoplasma groups (Wei et al. 2007).

So far, phytoplasmas belonging to 16SrI (Aster yellows) and 16SrXII (Stolbur) groups in Europe (Duduk et al. 2008, Satta 2016), the 16SrV (Elm yellows) group in Israel (Weintraub and Orenstein 2004), the 16SrII (Peanut witches'-broom) group in Saudi Arabia (Omar 2017), and the 16SrVI (Clover proliferation) group in the USA (Lee et al. 2006) have been reported to infect carrot in fields. In addition, carrots and weeds (Convolvulus arvensis and Daucus carota wild) were found to be infected with the 16SrXII group in Hatay province located in the Eastern Mediterranean Region of Turkey (Sertkaya 2014).

Insect vectors are considered the main reason for quick phytoplasma spread (Hogenhout et al. 2008). Human activities such as transportation and the use of infected plant propagation materials at the initial stage of cultivation also have been associated with the geographical transmission of phytoplasma groups (Al-Sadi et al. 2012, Mazraie et al. 2019). Several studies showed that phytoplasma groups can also be transmitted via seeds in various plant species, including carrots (Calari et al. 2011, Khan et al. 2002, Satta et al. 2019). In addition, perennial crop plants and weeds or wild plants could serve as reservoirs (Duduk et al. 2018, Kumari et al. 2019). Thus, weeds and wild plants are of utmost importance to understand epidemiological aspects of various diseases associated with phytoplasmas (Banzato et al. 2021).

Various phytoplasma groups have been reported to cause substantial yield reduction on carrot production in several countries, but, in the case of Turkey, the knowledge on both their presence and phylogenetic positions is rather limited. Moreover, there is still a huge gap in our understanding of inoculum sources of phytoplasma diseases. In this study, we investigated the potential of carrot seed and reservoir weed species as sources of phytoplasmas that occurred in carrot fields in the Ankara and Konya provinces.

MATERIALS AND METHODS

Weed and carrot seed sampling

Symptomatic and asymptomatic seven weed species belonging to five different families were sampled from carrot fields and their surrounding areas in Beypazarı and Nallıhan districts of Ankara and Meram district of Konya, Turkey, during growing seasons between April 2018 and September 2020. Nineteen weed samples were collected, including five *Amaranthus retroflexus* (fam: Amaranthaceae), seven *Sinapis arvensis* (Fam: Brassicaceae), three *Conium maculatum* (Fam: Apiaceae), one *Bifora radians* (Fam: Apiaceae), one *Daucus carota* wild (Fam: Apiaceae), one *Medicago sativa* (Fam: Fabaceae), and one *Fumaria officinalis* (Fam: Papaveraceae). Symptoms such as reddening of leaf tips, mosaic, and vein clearing were noted on a few of them while most others were symptomless. Seeds of eight widely grown carrot cultivars were also obtained from farmers during the surveys. Seed samples were taken into 50 ml glass tubes and stored at 4 oC until sowing.

For the germination of carrot seeds, a sterile and insect-free growing environment was provided under greenhouse conditions. Since the number of carrot seeds was quite limited, only approximately 200-300 seeds per cultivar were sown on two sets of pots. First, sterilized soil was placed into 9 cm width sterile pots, and then the seeds were sown on the shallow upper surface of the soil. The sown seeds were regularly irrigated with distilled water. The seedlings, including their roots, were harvested at the cotyledons leaf stage (before the true leafing stage) and then prepared for nucleic acid isolation.

Total nucleic acid (TNA) isolation

TNA was isolated from the shoot tissues of 19 weed samples and 32 freshly harvested seedlings of eight carrot cultivars at the cotyledon stage using CTAB protocol with some modifications (Li et al. 2008). Freshly harvested seedlings were cleaned of soil particles using 70% alcohol and sterile water. Two hundred mg tissue from each weed sample and 1000 mg tissue from seedlings of each cultivar were homogenized to form the isolation starting materials. The tissue was well crushed in CTAB solution buffer (2%

CTAB, 2% PVP-40, 100 mM Tris-HCL pH 8.0, 0.5 M EDTA, 1.4 M NaCl, 0.02% MCE) at a ratio of 1:5 (w:v) and then incubated at 65 oC for 20 min before centrifuged at 17,000 rpm for 10 min. The supernatant (850 µl) was treated with an equal volume of chloroform/isoamyl alcohol (24:1) and then centrifuged at 17,000 rpm for 20 min. The fluid phase on the upper surface was transferred to a sterile tube and treated with 0.7 volume of isopropanol, and the nucleic acid pellet obtained after centrifugation was washed with ice-cold 70% alcohol. The quality and purity of the genomic DNA obtained after the pellet was dissolved with nuclease-free water were measured using NanoDrop 2000 (Thermo Scientific, U.S.A). TNA was stored at -20 oC until further used in the nested-PCR assay.

Nested-PCR amplification

Nested-PCR experiments were done using R16mF2/R16mR2 or R16F2n/R2 primer pairs (Gundersen and Lee 1996) followed by fU5/rU3 (Lorenz et al. 1995) primer pairs to identify phytoplasma from its 16S rRNA gene. DNA concentrations from within TNAs for the first PCR reaction were made using NanoDrop 2000 (Thermo Scientific, U.S.A) and about 0.05 µg of gDNA was used for each reaction tube. The first amplification products from Nested-PCR assays were diluted 1:30 with nuclease-free water before being utilized in subsequent reactions.

The reactions were implemented in 25 µl PCR tubes containing 0.05 µg genomic DNA (first PCR) and 1 µl of 30 µl diluted PCR product (second PCR), 1 µl of 10 mM dNTPs, 0.2 µl of 25 mM MgCl2, 1 µl of 10 mM phytoplasma universal primers, 2.5 µl of 10X PCR buffer, and 0.25 U of Taq DNA polymerase (5 U/µl) (Ampliqon, Denmark). The amplification conditions were carried out as stated by Gundersen and Lee (1996). The PCR products were separated on 1.2% agarose gel stained with ethidium bromide. The amplicon's size was measured using a UV light imaging system (Genegenius, U.K.).

Phylogenetic analysis and sequencing

If there were more than one positive nested-PCR amplification result from the same cultivar in the seedling experiments, two results were chosen for nucleotide sequencing, and one sample was chosen when there was only one positive result. The Sanger method was applied by a commercial firm (BM lab, Ankara, Turkey) to reveal bidirectional sequencing of a total of 16 PCR products from four weed samples and seedlings. BLAST (Basic Local Alignment Search Tool) analysis of the NCBI (the National Center for Biotechnology Information) was used to confirm the nucleotide (nt) data obtained by sequencing with those of other phytoplasma isolates in the GenBank. The sequences were then submitted to the NCBI database under acc. nos. MZ463005-MZ463020.

The sequences obtained from weeds and seedlings were aligned with other sequences of different phytoplasma groups from across the world using ClustalW in MEGAX software (Kumar et al. 2018). The best models for nt substitution were selected using the lowest BIC (Bayesian Information Criterion) scores. The phytoplasma phylogenetic tree was generated by the Maximum likelihood method (Felsenstein 1981) with 1000 bootstrap repetitions using the Tamura-Nei parameter model (TN93) (Tamura and Nei 1993)+Gamma distributed (G). The *Spiroplasma citri* 16S rRNA gene, isolate Qualubia (acces no AM157769) was used as the out-group.

In silico PCR-RFLP analyses

Computer-simulated PCR-RFLP (the restriction fragment length polymorphism) analyses were performed using the Snapgene software (GSL Biotech; http://www.snapgene.com) to improve the accuracy of phytoplasma classification based on partial of the 16S rRNA gene sequences. For this purpose, a total of 16 nt sequences obtained from carrot seedlings and weeds were digested in silico with RsaI enzyme, among 17 RFLP enzymes (*RsaI*, *SspI*, and *TaqI*, *BfaI*, *BstUI* (Tha*I*), *AluI*, *BamHI*, *DraI*, *HinfI*, *HpaI*, *EcoRI*, *HaeIII*, *HhaI*, *Sau3AI* (*MboI*), *HpaII*, *KpnI*, *MseI*) that were widely used in the determination of phytoplasma groups (Lee et al. 1998). A virtual 4% agarose gel electrophoresis image was plotted to the computer screen automatically after in silico restriction digestion. In addition, the partial sequences of 16Sr gene fragments of Ca. P. trifolii 16SrVI-A (acces no AY390261), Brinjal little leaf (BLL) 16SrVI-D (acces no EF186820), Oenothera phytoplasma 16SrI-B (acces no M30790), Aster yellows phytoplasma (ACLR-AY) 16SrI-F (acces no AY265211), Clover phyllody phytoplasma (CPh) 16SrI-C (acces no AF22065), Elm yellows phytoplasma (EY1) 16SrV-A (acces no AY197655), Peanut witches-broom phytoplasma 16SrII-A (access no L33765), Ca. P. solani (Stolbur) 16SrXII-A (access no AJ964960), and Australian grapevine yellows 16SrXII-B (access no L76865) isolates were used in silico analyses to compare the patterns obtained from RFLP analyses with Turkish weed and seedlings isolates.

RESULTS

Symptomology of seedlings and weeds

No symptom was observed on 15 weed samples. However, vein clearing, reddening at the leaf tips, yellowing, and severe reddening/purpling were observed on one of each *S. arvensis*, *C. maculatum*, *M. sativa*, and *D. carota* wild, respectively (Figures 1 and 2, Table 2). There was also no symptom caused by phytoplasma observed on all seedlings of eight cultivars planted on 16 pots up to harvest time at

the cotyledon stage. However, germination rates of cultivars were different, particularly, seeds of a local cultivar (TS1) germinated rate below 50% and resulted in the death of some seedlings (Table 1). In addition, germination rates of other commercial carrot seeds differed between 70% and over 90% (Table 1 and Figure 3).

Figure 1. (a) redding of leaf tips symptoms on *Conium maculatum* (b) severe redding or purpling symptoms on *Dacus carota* wild

Figure 2. (b) Vein clearing symptoms on Sinapis arvensis (b) yellowing symptoms on Medicago sativa

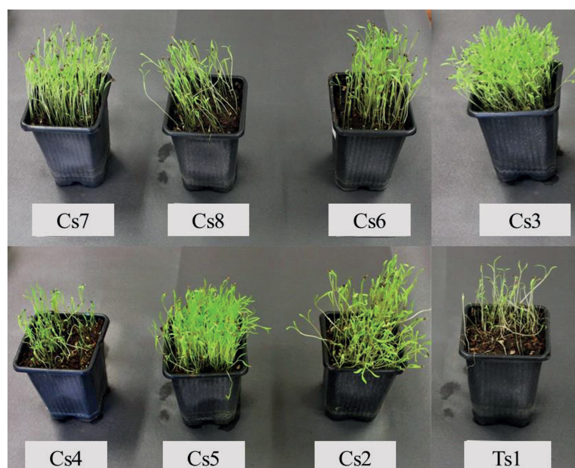

Figure 3. Germination performances of different carrot seed varieties (Cs: Commercial seed, Ts: Traditional seed)

Table 1. Germination and infection status of carrot seedlings germinated in a controlled environment

Carrot seed cultivars	Total no of DNA samples	Total no of infected samples	Germination results	Phytoplasma infection rRNA
TS1*	4	4	Germination rate below 50% and dead seedlings	16SrVI
CS2**	5	3	Germination over 80%	16SrVI
CS3**	8	5	Germination over 90%	16SrVI
CS4**	3	3	Germination about 70%	16SrVI
CS5**	2	0	Germination over 90%	-
CS6**	3	1	Germination over 80%	16SrVI
CS7**	5	3	Germination over 80%	16SrVI
CS8**	2	1	Germination about 70%	16SrI
Total	32	20		

*TS: Local seed CS**: Commercial Seed

Table 2. Symptoms observed in weeds and status of phytoplasma infections

Weed sample	Total number of sample	Total no of infected sample	Symptoms	Phytoplasma infection rRNA
Amaranthus retroflexus	5	0	5=asymptomatic	-
Sinapis arvensis	7	1	5=asymptomatic 1=vein clearing 1=chlorotic discoloration	16SrVI
Conium maculatum	3	1	2=asymptomatic 1=redding of leaf tips	16SrVI
Medicago sativa	1	1	1=yellowing	16SrVI
Fumaria officinalis	1	0	1=asymptomatic	-
Daucus carota wild	1	1	1=severe redding or purpling	16SrVI
Bifora radians	1	0	1=asymptomatic	-
Total	19	4		

TNA isolation and molecular assays

TNAs were successfully obtained using the CTAB nucleic acid extraction protocol, which had been modified to also obtain genomic DNAs. Spectrophotometric measurements confirmed the quality and purity of the obtained TNAs as suitable templates for PCR studies. Successful amplifications were produced in the nested-PCR experiments using fU5/rU3 (Lorenz et al. 1995) primer pairs and the expected 883 bp long amplicons were obtained (Figure 4). Positive findings were acquired from 20 out of 32 carrot seedlings and 4 out of 19 weed samples. Tables 1 and 2 details the molecular detection results for both seedlings and weeds.

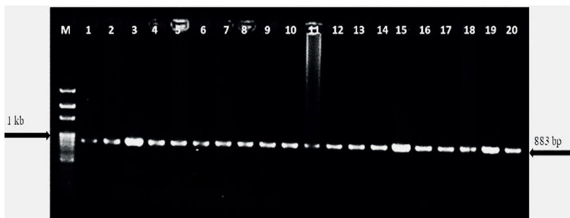

Figure 4. Image of fragments of about 883 bp in agarose gel obtained from weeds and carrot seedlings PCR studies using fU5/rU3 primers (1:*Sinapis arvensis*, 2:*Dacus carota*, 3:*Medicago sativa*, 4:*Conium maculatum* , 5:Ts1a, 6:Cs2c, 7:Cs3c, 8:Cs3a, 9:Cs2e, 10: Ts1b, 11:Cs4a, 12:Cs7c, 13:Cs6a, 14:Cs7b, 15:Cs8c, 16:Cs4c, 17:Ts1c ,18:Cs3b , 19: Cs7a, 20:Positive control (M: Marker 100 bp; Solis Biodyne, Estonia)

Phylogenetic analysis and sequence similarities

To compare the sequences of the partial fragment of the 16S rRNA gene region, a total of 16 isolates obtained from carrot seedlings and weeds were analysed with other phytoplasma strains available in the GenBank. The nt sequence of CS8c isolate obtained from a commercial cultivar was 99.65% similar to an Iranian isolate 'Bajgah periwinkle little phytoplasma' (acc. no. DQ266089) of 16SrI 'Aster Yellows' group. The rest of the isolates (fifteen out of 16) showed 99.78-99.89% nt sequence identities with 'Eggplant phyllody phytoplasma' (acc. no. MT240537), '*Candidatus* Phytoplasma trifolii-grape5' (acc. no. MK392485), and 'Brinjal Leaf Phytoplasma-BLL' (acc. no. MT071396) of 16SrVI 'Ca. P. trifolii' group.

The phylogenetic tree was constructed based on an 883 bp fragment of the 16S rRNA using sequences of different phytoplasma groups/subgroups herein obtained and also retrieved from the GenBank (Figure 5). Four weed and eleven seedlings (from both local and commercial cultivars, Table 1) isolates were clustered within the 16SrVI group in the phylogram (Figure 5). On the other hand, the CS8c isolate obtained from a commercial carrot cultivar was clustered in the 16SrI group (Figure 5). Major clades in which the weed and seedling isolates clustered were supported by high bootstrap reliability values (>90).

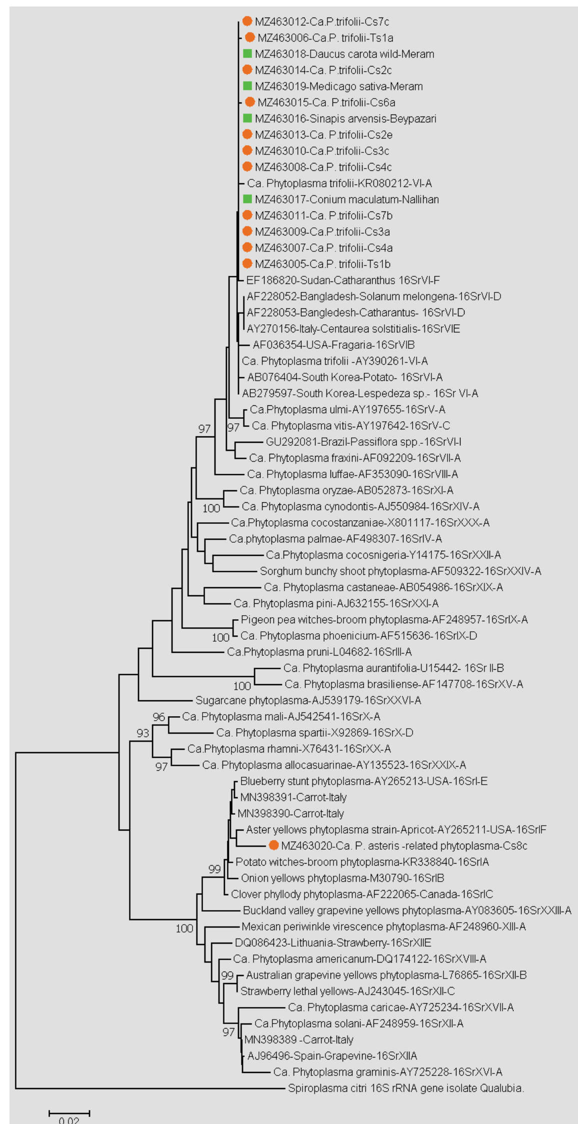

Figure 5. Phylogenetic cladogram constructed by Maximum likelihood test of nt of partial 16S rRNA gene. Fifty-nine isolates were applied in the 16S rRNA comparison. Turkish weed and seedling isolates are marked with circle and square symbols. Bootstrap values on each branch were supported by 1000 replicates; only values greater than 90% were shown. The *Spiroplasma citri* 16S rRNA gene, isolate Qualubia (access no AM157769) was used as the out-group

As a result, the phytoplasma isolates obtained in this study were clustered in the same evolutionary lineages with phytoplasma strains, namely 16SrVI and 16SrI strains, showing the monophyletic feature. Nt similarity and phylogenetic analyses also suggested that the 15 isolates in 16SrVI could be more specifically classified into the 16SrVI-A subgroup while the CS8c isolate has belonged to the 16SrI-F subgroup.

In silico PCR-RFLP

The phylogram and nt similarity results were supported by the in silico RFLP analysis. After comparing computer-

simulated PCR-RFLP analysis of virtual patterns of the partial 16S rRNA gene of sixteen isolates was determined that distinctive RFLP profiles with *RsaI* enzymes according to reference strains. The *RsaI* enzyme-digested profiles of fifteen isolates identified as related to the 16SrVI and 16SrI groups according to their nt similarity ratios were consistent with 16SrVI-A/D and 16SrI and were different from other references (16SrI-B/F/C, 16SrV-A, 16SrII-A, and 16SrXII-A/B) (Figure 6). The RFLP virtual pattern of the other 16SrI group-associated Cs8c isolate with the *RsaI* enzyme was consistent with the 16SrI-F subgroup, whereas it was different with the 16SrI-B/C subgroup and other groups/subgroups (16SrVI-A/D, 16SrV-A, 16SrII-A, and 16SrXII-A/B) (Figure 6).

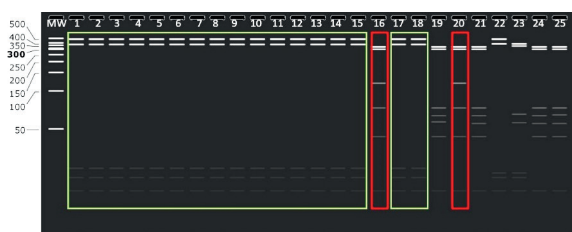

Figure 6. Virtual RFLP gel (4.0%) patterns from in *silico* digestion of partial 16S rRNA fU5/rU3 amplicons of phytoplasmas infecting carrot seedlings and weeds with *RsaI* restriction enzyme. The green rectangles demonstrate the digestion pattern of the 16SrVI-A/D reference strains. The red rectangles demonstrate the digestion pattern of the 16SrI-B/F/C reference strains. [1: Cs4c, 2: Cs7b, 3: Cs7c, 4: Cs4a, 5:Ts1a, 6:Cs2c, 7:Cs3c, 8:Cs3a, 9:Cs2e, 10: Ts1b, 11: *Conium maculatum*, 12: *Medicago sativa*, 13:Cs6a, 14: *Dacus carota*, 15:*Sinapis arvensis* 16: Cs8c] Weed and seedling isolates, [17] 16SrVI-A (AY3902619), [18] 16SrVI-D (EF186820), [19] 16SrI-B (M30790), [20] 16SrI-F (AY265211), [21] 16SrI-C AF222065), [22] 16SrV-A (AY197655), [23] 16SrII-A (L33765), [24] 16SrXII-B (L76865), [25] 16 SrXII-A (AJ964960). (MW; Qiagen, Gelpilot 50 bp ladder)

DISCUSSION

This study aimed to reveal seed and weeds as inoculum sources that play crucial roles in the spread of phytoplasma diseases causing symptoms such as redness/browning, yellowing, and reduced root quality which were widely observed in the largest carrot growing areas of Turkey (Ankara and Konya).

Transmission of phytoplasmas through seeds was somewhat a controversial topic and has long been considered impossible due to the lack of a direct connection between the phloem system and embryos (Menon and Pandalai 1960). However, it was later proved for the first time by Khan et al. (2002) that seeds of phytoplasma-infected symptomatic alfalfa mother plants transmitted this pathogen. Since then, there have been reports of seed transmission of phytoplasmas in other vegetables, such as tomato and winter oilseed rape (Calari et al. 2011), as well as *Brassica napus* (Satta et al. 2019) at the seedling stage. The presence of phytoplasmas on carrot seeds was first demonstrated by Carminati et al. (2019), and it was confirmed that these seeds were contaminated with the 16SrI 'Aster Yellows' group. In a later study, Satta et al. (2020) found that seedlings obtained from carrot seeds were infected at the cotyledon stage with one of the groups 16SrI and 16SrXII, and also seedlings of a batch belonging to the same group were infected with both groups of phytoplasmas. Therefore, this present study confirmed 16SrI group infection, while also detecting the 16SrVI group in the seedlings of carrot cultivars for the first time. The two phytoplasmas groups were not found together in any infected seedlings grown from the same cultivar according to our observation.

Abnormalities and malformations in the floral organs and fruits of various species infected with phytoplasmas resulted in the formation of seeds with reduced viability (McCoy et al. 1989). Accordingly, seed production in infected mother plants was affected both quantitatively and qualitatively due to the presence of phytoplasmas (Satta et al. 2019). More importantly, it has been reported that phytoplasma infection affects the expression of some flower development genes in the whole flower meristem of tomato and hydrangea plants (Himeno et al. 2011, Kitamura et al. 2009, Pracros et al. 2006). Therefore, late infections usually still produce a normal number of seeds, but these seeds could be infected and thus give the pathogen a greater chance of transmission, conversely, early infection causes such serious changes in the mother plant that seed production and viability of the seeds as next-generation planting materials are highly reduced (Satta et al. 2020). In our study, while the germination rates and development of seedlings of commercial cultivars stayed at a normal level, very weak germination, growth, and even death of some seedlings were observed on a non-commercial/local red carrot cultivar (named TS1) which is regularly cultivated and harvested under uncontrolled field environment every year by farmers themselves. This could indicate that the TS1 mother plants were infected by phytoplasma at the early growing season and exposed to the pathogen for a long time before flowering.

At least 43 weed species with phytoplasma infections have been documented from around the world (Mall et al. 2010). The most prominent symptoms on infected plants were the proliferation of axillary shoots, small leaves, extensive chlorosis, witches' broom, and yellowing (Mall et al. 2010). The phytoplasmas found in weeds around the world mostly belong to the 16SrI, 16SrII, 16SrXI, 16SrXII, and 16SrXIV groups, but some members of the 16SrIII, 16SrIV, 16SrV, 16SrVI, 16SrVII, 16SrIX, 16SrX, and 16SrXXIX groups have

also been identified (Duduk et al. 2018). Also, studies have shown that while *A. retroflexus* weed is infested with the 16SrI group, and 16SrV-B and 16SrXII-A subgroups (Credi et al. 2006, Wu et al. 2010, Yang et al. 2011), phytoplasmas have not been found in *F. officinalis* and *B. radians* weeds. Similarly, phytoplasma infection was not detected in any of the asymptomatic *A. retroflexus*, *F. officinalis*, and *B. radians* weeds collected from inside and around carrot fields in our study. On the other hand, this present study revealed that symptomatic *M. sativa*, *S. arvensis*, *D. carota* wild, and *C. maculatum* weeds were infected by the 16SrVI group. Other studies determined that M. sativa was infected with 16SrII-C/D and 16SrVI-A subgroups, and 16SrXII groups (Credi et al. 2006, Esmailzadeh Hosseini et al. 2016a, 2016b). Infections by 16SrIX-C 'withes`s broom', 16SrI-(B/AJ), and 16SrXII-A phytoplasma subgroups have been reported in wild plants *S. arvensis*, *C. maculatum*, and *D. carota* wild, respectively (Casati et al. 2016, Fernández et al. 2020, Sertkaya 2014). Therefore, according to our best knowledge, this was the first report of the presence of 16SrVI group phytoplasma in the *S. arvensis*, *C. maculatum*, and *D. carota* wild.

According to Satta et al. (2020) and the results of our study, no symptomological finding could be related to phytoplasma infected seedlings. However, poor growth and even death were observed on seedlings germinated from a local cultivar. In future studies, factors affecting the germination performance of infected seeds should be evaluated in detail, both genetically and physiologically. Insect vector's role in epidemiology also needs to be investigated as they could spread phytoplasma further from infected weeds and cultured plants under field conditions, which in turn could accumulate the pathogen population in cultivation areas, thus increasing future sources of inoculum.

Seedlings were obtained from carrot seeds in the cotyledon leaf stages, and the phytoplasma contamination of these seeds was indirectly revealed by conventional molecular techniques in a short period of time. The methodology that may use in the detection of phytoplasmas from seedlings obtained from seeds is recommended to be applied to different vegetable seeds, an algorithmic method should be developed by determining certain parameters to detect the effects of infections on seed germination. Furthermore, population genetic structures should be revealed using phytoplasma genes in the future to better understand the spread of phytoplasmas from one place to another by seed and their adaptation to weeds in nature.

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ÖZET

Havuçta şiddetli sararma ve kızarıklık belirtileri ile ilişkili fitoplazma inokulum kaynakları Ankara ve Konya illerinde araştırılmıştır. Bu çalışma kapsamında bölgelerde yoğun olarak ekimi yapılan yedi havuç çeşidinin tohumlarında ve tarlalarda yer alan yabancı otlarda 16SrVI ve 16SrI ilişkili fitoplazmaların varlığı tespit edilmiştir. Konvansiyonel moleküler yöntemler (nested-PCR) kullanılarak dizi analizleri ile fitoplazma gruplarının varlığı doğrulanmış ve filogenetik analizler ile desteklenmiştir. Ayrıca elde edilen nükleotid dizileri, *in silico* PCR-RFLP analizi ile referans fitoplazma dizileri ile karşılaştırılmıştır. *Daucus carota* wild, *Medicago sativa*, *Conium maculatum* ve *Sinapis arvensis* yabancı otlarında 16SrVI (Clover proliferation) fitoplazma grubu ile enfeksiyon belirlenmiştir. Ayrıca yedi havuç çeşidinin tohumlarından çimlenen fidelerde 16SrVI ve 16SrI (Aster yellows) fitoplazma grupları tespit edilmiştir: Biri yerel kırmızı havuç çeşidi ve altısı ticari olarak üretilmiş çeşitlerdir. Bilgilerimize göre elde edilen bulgular; 16SrVI grubu ile enfekte olmuş havuç tohumlarının ve *S. arvensi*s, *C. maculatum* ve *D. carota* wild'da 16SrVI grubunun varlığının ilk raporudur.

Anahtar kelimeler: fitoplazma, fide, yabancı ot, Nested-PCR, *in silico* analiz, filogenetik

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