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MOLECULAR CHARACTERIZATION OF *Tomato yellow leaf curl virus* AND *Fusarium oxysporum* formae speciales AND RACES OF TOMATO AREAS IN NORTHERN CYPRUS

Ayşegül Çolak Ateş[1]✉, Hakan Fidan[2], Bahar Gökhan[3]

[1] Biological Control Research Institute, Kısla Street, 01321 Yuregir, Adana, Turkey
[2] Akdeniz University, Faculty of Agriculture, Plant Protection, Antalya, Turkey
[3] Agriculture Research Institute, Lefkoşa, Northern Cyprus

ABSTRACT

Fusarium oxysporum (FO) and viruses have caused wilt, root, crown rots, mosaic, yellowing and curling on tomato plants and have resulted economic yield losses on tomato production areas at Northern Cyprus (NC) in 2011–2015 years. Typical FO symptoms showing greenhouse and open field areas used for collecting 62 plants and suspected *Tomato yellow leaf curl virus* (TYLCV) of 76 tomato plants have been studied respectively. In the researches, four different primers [uni,sp13,sp23,sprl] were used to determine the formae specialis and races of 62 isolates of FO isolates from different locations. PCR analyse studies have revealed that 81% of collected samples were *Fusarium oxysporum* f.sp. *lycopersici* (FOL) and 19% of them were *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL). Additionally, further PCR analyses have identified that 37% of FOL samples were race 1, 15% were race 2 and 29% were race 3 identified respectively. The different samples of 76 tomato plants were tested with specific primers in PCR amplifications. Their results determined that the strains TYLCV-Israel, TYLCV-Sicilia, TYLCV-Mild of TYLCV found. The molecular techniques have suggested that Israel, Sicilia and Mild strain of TYLCV were present in the tomato production areas at NC. The TYLCV races with single or mixed infections and *Fusarium oxysporum* formae specialis and races are able to identify in molecular techniques in not only accurately but also reliably.

Key words: tomato, *Fusarium oxysporum*, *Tomato yellow leaf curl virus*, PCR, strain

INTRODUCTION

Tomato plant, *Solanaceae* family *Lycopersicum* genus, due to its important vitamin and mineral content, is known to have a positive impact on human health. Tomato cultivation is being promoted by increase in its consumption both as a fresh vegetable and in processed food. In 2016, 7.959 tons of tomato product were harvested in greenhouses-field and on 1537 area in Northern Cyprus (NC) [KKTC Tarım ve Doğal Kaynaklar Bakanlığı 2017]. Although tomato cultivation is intensive in the region, production is insufficient in certain periods and tomato import is required. The main factors behind this are soil borne fungal and viral diseases.

In greenhouse tomato cultivation, soil-borne pathogens lead to significant problems due to the cultivation of a single species in a certain field. In tomato culti-

✉ aysegulcolak@hotmail.com

vation, *Fusarium oxysporum* is the most economically significant plant-root disease among the soil-borne fungi. The species has two pathogenic special forms that are morphologically different and lead to wilt and crown – root rot disease: *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis-lycopersici*. These soil-borne pathogens attack the roots, crown and lower parts of the plant stem, causing deterioration and rotting in these tissues and wilt diseases [Laine et al. 1999, Balmas et al. 2005]. The most accurate diagnosis of this two disease, which cannot be easily distinguished based on morphological properties, is conducted with specific molecular markers [Çolak and Biçici 2013]. Determination of active *Fusarium* species and strains in the region is very important to determine the effective control methods against the disease. Identification of these two pathogenic forms of *F. oxysporum* would make it possible to identify the varieties that are resistant to the particular pathogenic form [Çolak Ateş et al. 2019].

It was reported that *Tomato yellow leaf curl virus* (TYLCV) was associated with *Bemisia tabaci* outbreaks in the 1940s. In 1964, in contrast to other known plant viruses in Israel, it was reported that it was persistent transported by vector white fly (*Bemisia tabaci*) and was not transported via seed vector in several previous studies. It is known that the virus, which is manifested by upward curls in small yellow and top shoots in the main host in the tomato plant, is known to harm the leaves more than the fruit than the fruit, for which it results in a reduction in size and quantity. Although wild hosts do not suffer any symptoms, bean, lisianthus, petunia, poisonberry, eggplant, pepper, and wild tomato species are considered significant hosts. It is a single or two-part DNA virus, with genome consisting approximately of 2800 nucleotides. Although having such a small genome, it has Asian and Mediterranean strains and there exists differences between these strains. Virus can be effectively transported to other plants by vector *Bemisia tabaci* Biotype B and Q. TYLCV is encountered in all georgaphic areas where the white fly problem exists [Czosnek 2007, Abhary et al. 2006, Fidan et al. 2011, Çolak Ateş et al. 2019]. Characteristic symtom of the disease are scrubs, hovewer, scrubbiness are more common in young plants. Infected plants create few flowers which next form underdeveloped fruits. Product losses can be as high as 80-100% [Moriones and Navas 2000].

Diverse strains of TYLCV were reported in different parts of the world. The most common among these strains is the *Tomato yellow leaf curl Israel virus* (TYLCV-Is). It is followed by the *Tomato yellow leaf curl Sicilia virus* (TYLCSV-Sic), *Tomato yellow leaf curl Mild virus* (TYLCV-Mld) and *Tomato yellow leaf curl Sardinia virus* (TYLCV-Sa), *Tomato yellow leaf curl Morocco virus* (TYLCMOV), *Tomato yellow leaf curl Malaga virus* (TYLCMalV), *Tomato yellow leaf curl Axarquia virus* (TYLCAxV), *Tomato yellow leaf curl*-China (TYLCCNV) and *Tomato yellow leaf curl* -Thailand (TYLCTHV) [Anfoka et al. 2005].

In the present study, the symptomatologic and molecular diagnosis of the *Fusarium* formae speciales and races and *Tomato yellow leaf curl virus*, which cause paleness and root-root collar rot in plants in different regions of NC with greenhouse and field tomato cultivation, and their identification and prevalence were revealed.

MATERIALS AND METHODS

Fungus and Virus Isolation from tomato cultivation area. In a total of 1.477.000 m^2 in different regions of NC, where greenhouse and field tomato cultivation were conducted, samples were collected from tomato plants that exhibited the typical viral symptoms of TYLCV, such as paleness, browning in vascular bundles, yellowing on leaf edges, curling, scrubbiness, non-fructification or small fruit development.

In the present study, 62 FO isolates, derived from different regions in NC, were studied for *Fusarium oxysporum* (FO) formae speciales infection and determining their strains. These isolates were obtained from plants with typical disease symtoms, which had typical paleness and root-root collar rot due to FO, from Gazimağusa, Güzelyurt, Girne, Lefkoşa ve İskele regions between the years 2011 and 2015, where greenhouse and field tomato cultivation were common. 2–3 mm samples have been taken with a clean scalpel from pathologically changed and healthy tissues from the root, root collar and the stems. Samples were surface sterilized by washing in a sterile beaker with 1% sodium hypochlorite (NaOCI) solution for 2 to 3 min. Thereafter, samples were washed twice with sterile distilled water to remove the NaOCI resi-

dues and transferred to sterile blotting paper to remove excess water. The dried tissue samples were applied in 9.0 cm Petri dishes that contain Potato Dextrose Agar (PDA) medium. Petri dishes were incubated at 24°C for a week [Katan et al. 1997]. Consequent to the incubation process, the *F. oxysporum* isolates were examined microscopically for colony development, color, phialide, chlamydospore, micro, and macro conidia characteristics and the single-spore isolation was completed. All fungal isolates from different regions were kept at +4°C for use in DNA and PCR studies [Çolak and Biçici 2013].

The present study was conducted in the tomato cultivation areas, with the purpose to determine the strains in NC that cause the *Tomato yellow leaf curl virus* disease in tomato plant. During the surveys, both, tomato plants that were suspected to be contaminated by the virus, and the whiteflies existing on these plants, were collected 76 samples from tomato plants. And then, the collected samples were tested via polymerase chain reaction (PCR) method using strain-specific primers to determine the strains and recombinants of TYLCV. The obtained PCR products were sequenced, and their phylogenetic classification was completed in order to verify the accuracy of these strains.

DNA Isolation. The DNA Purification Mini Kit (Thermo Scientific GeneJET Plant-K0792) was used for total genomic DNA isolation of the *Fusarium oxysporum* (FO) and TYLCV isolates. For this purpose, the isolates were incubated for 7 to 10 days in PDA for FO isolates and micelles were lyophilized through a double-layer gauze. 100 mg of the obtained micelles were used to obtain the total genomic DNA isolation via the Mini Kit.

For the TYLCV isolates, the DNA isolations were conducted for 100 to 400 mg of leaf samples retrieved from tomato plants that exhibited typical TYLCV symptoms (such as yellowing that starts from the leaf edges, spooning, leaf curling, discoloration, scrubbiness) [Anfoka et al. 2005]. Concentration of DNA was adjusted 40 ng with the help of spectrophotometer in order to determine the presence of all DNAs obtained from FO and TYLCV isolates that belonged to the isolates of the regions, the controls were conducted through a 2% Agarose Gel Electrophoresis study. All DNA samples were stored at –20°C for further use in PCR studies.

PCR conditions in the diagnosis of *Fusarium oxysporum* formae speciales. In the present study, uni, sp 13, sp 23, and sprl primers were used for PCR studies, based on Hirano and Arie [1996] in order to determine the *Fusarium oxysporum* formae speciales and strains in 62 FO isolates (Tab. 1). A total of 25 µl reacion mixtruse contained with 18 µl Master mix (1.25 µl dNTP, 5 µl buffer, 2 µl $MgCl_2$, 0.2 µl Taq DNA polymerase, 9.55 distilled water), 2 µl Primer (1 µl F + 1µl R) and 5 µl genomic DNA (10 ng). The reactions were operated under sterile conditions and on ice [Çolak and Biçici 2013].

PCR conditions were programmed as 5 min and 45 cycles of 1 min at 61°C and 2 min at 72°C and 45 cycles and a final expansion of 10 min at 72°C [Hirano and Arie 2006]. PCR products were separated on 2% agarose gel, which was then placed in 0,5 µg/ml solution of Ethidium Bromide and visualized under UV light. Gel image was analysed in accordance with Hirano and Arie [2006]. The PCR studies were repeated twice.

PCR conditions in the diagnosis of TYLCV. The specific primers developed by Anfoka et al. [2008] were used in the strain diagnosis studies of TYLCV isolates (Tab. 2, 3 and 4). Two different multiplex PCR combinations were used for the identification of the strains. The Primer Set 1 (TYLCV1, TYLCV2, TYLCV3, TYLCV4, TYLCV5) was optimized for 1st Multiplex PCR by 35 cycles and conditions of the program can be putted in the bracket (35 cycles: 00'30" in 95°C, 01'00" in 52°C, 01'00" in 72°C; followed by 10'00" in 72°C. The Primer Set 2 (TYLCV4, TYLCV5, TYLCV6, TYLCV7, and TYLCV8) was used for 2nd Multiplex PCR (35 cycles: 00'30" in 95°C, 00'45" in 62°C, 01'00" in 72°C; followed by 10'00" in 72°C.

Sequence Analysis for the TYLCV. Three different isolates of each strain, positively classified via PCR, have been subjected to a sequence analysis. New PCR reaction was performed in volume of 50 µl using the Finnzymes' Phusion® High-Fidelity DNA Polymerase enzyme. 10 µl of each product of reaction were checked on agarose gel. The remaining 40 µI was sent to the Senrtromer company and nucleotide sequences of the reproduced region were obtained. The obtained from the BioEdit (BioEdit 7.2.6 Sequence Alignment Editor) software. The protein sequence was examined using the ExPASy (https://web.expasy.org/cgi-bin/

Table 1. Nucleotide sequences and band size of primers synthesized for PCR analysis

Primer	Base sequence (5'3')	Fragment (bp)	Evaluation
uni-f	ATCATCTTGTGCCAACTTCAG	670–672	*Fusarium oxysproum*
uni-r	GTTTGTGATCTTTGAGTTGCCA		
sprl-f	GATGGTGGAACGGTATGACC	947	FOL 1 ve 3
sprl-r	CCATCACACAAGAACACAGGA		
sp13-f	GTCAGTCCATTGGCTCTCTC	445	FOL 2 ve 3
sp13-r	TCCTTGACACCATCACAGAG		
sp23-f	CCTCTTGTCTTTGTCTCACGA	518	FORL
sp23-r	GCAACAGGTCGTGGGGAAAA		

Table 2. Primers and base sequences used to determine TYLCV strains

Primer	Base sequence (5'3')
TYLCV1	TTTTATTTGTTGGTGTTTGTAGTTGAAG
TYLCV2	ATATTGATGGTTTTTTCAAAACTTAGAAG
TYLCV3	ACGTAGGTCTTGACATCTGTTGAGCTC
TYLCV4	AAGTGGGTCCCACATATTGCAAGAC
TYLCV5	ATTGACCAAGATTTTTACACTTATCCC
TYLCV6	ATACTTGGACACCTAATGGCTATTTGG
TYLCV7	TGCCTTGGACA[A/G]TGGGG[A/G]CAGCAG
TYLCV8	TGGAAAGTACCCCATTCAAGAACATC

Table 3. Multiplex PCR primer combinations (Primer set1)

TYLCV strain	Primer	Fragment (bp)
Tomato yellow leaf curl virus-Israel(TYLCV-Is)	TYLCV3, TYLCV4	634 bp
Tomato yellow leaf curl Sardinia virus (TYLCSV-Sa)	TYLCV1, TYLCV2	433 bp
Tomato yellow leaf curl virus-Mild (TYLCV-Mld)	TYLCV5, TYLCV4	316 bp

Table 4. Multiplex PCR primer combinations (Primer set2)

TYLCV strain	Primer	Fragment (bp)
Tomato yellow leaf curl virus (TYLCV-Is)	TYLCV6, TYLCV7	543 bp
Tomato yellow leaf curl virus-Sicilia (TYLCV-Sic)	TYLCV8, TYLCV6	946 bp
Tomato yellow leaf curl virus-Mild (TYLCV-Mld)	TYLCV5, TYLCV4	316 bp

translate/dna2aa.cgi) software and the significance of these sequences was scrutinized. The sequences that accomplished all abovementioned steps were compared with the gene databases (NCBI, National Center for Biotechnology Information).

RESULTS AND DISCUSSION

In a total of 1.477.000 m^2 in different regions of NC, where greenhouse and field tomato cultivation were conducted, 577.000 m^2 (39.1%) of the 1.477.000 m^2 of tomato cultivation areas in different regions were found to be infected with *Fusarium oxysporum* and 689.000 m^2 (46,6%) were infected with *Tomato yellow leaf curl virus* (Tab. 5). In the present study, the highest *Fusarium oxysporum* infection in tomato cultivation areas was detected in Lefkoşa with 67.6%, followed by Famagusta, Kyrenia, Güzelyurt, and İskele. The most infected area with TYLCV symptoms was Girne (61%) with followed by Lefkoşa, Gazimağusa, Güzelyurt, and İskele (Tab. 5).

The set of four different primers (uni, sp 13, sp 23, sprl) was used for identifying the formae speciales and the strains of the 62 *Fusarium oxysporum* isolates collected from different regions of NC (Fig. 2). Based on the PCR studies, it was determined that *Fusarium oxysporum* f.sp. *lycopersici* [FOL] was was present in 81% of the plant samples and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) was responsible for root-root collar rot in 19% (Tab. 6). Within that 81% mentioned above was FOL1, FOL2 and FOL3, with prevalence 37%, 15% and 29% respectively (Tab. 6 and Fig. 1).

Pest control for the forms of *Fusarium oxysporum* is highly difficult since these forms have soil-borne diseases [Çolak and Biçici 2013]. In the context of the present region, such difficulty increases due to the inadequacy of infrastructure in terms of cultural processes such as irrigation, land leveling, drainage, and soil sterilization cause more complications for tomato cultivation. With respect to the reasons such as effective soil sterilization (solarization) in the region have

Table 5. *Fusarium oxysporum* and TYLCV presence rates in different regions of NC (2011–2015)

Location	Location area	Infected area			
		Fusarium oxysporum		TYLCV	
	da	da	%	da	%
Gazimağusa	271	140	51.7	110	40.6
Güzelyurt	474	210	44.3	165	34.8
Girne	574	270	47.0	350	61.0
Lefkoşa	111	75	67.6	60	54.1
İskele	47	12	25.5	4	8.5
Total	1477	577	39.1	689	46.6

Table 6. Formae speciales and races of *Fusarium oxysporum* isolates separation obtained by PCR

Location	FO isolate number	FOL1		FOL2		FOL3		FORL	
		isolate number	%	isolate number	%	isolate number	%	isolate number	%
Gazimağusa	12	4	33	2	17	4	33	2	17
Güzelyurt	17	6	35	1	6	7	41	3	18
Girne	21	8	38	3	14	6	29	4	19
Lefkoşa	8	3	43	2	29	1	14	2	14
İskele	4	2	29	1	14	0	0	1	57
Total	62	23	37	9	15	18	29	12	19

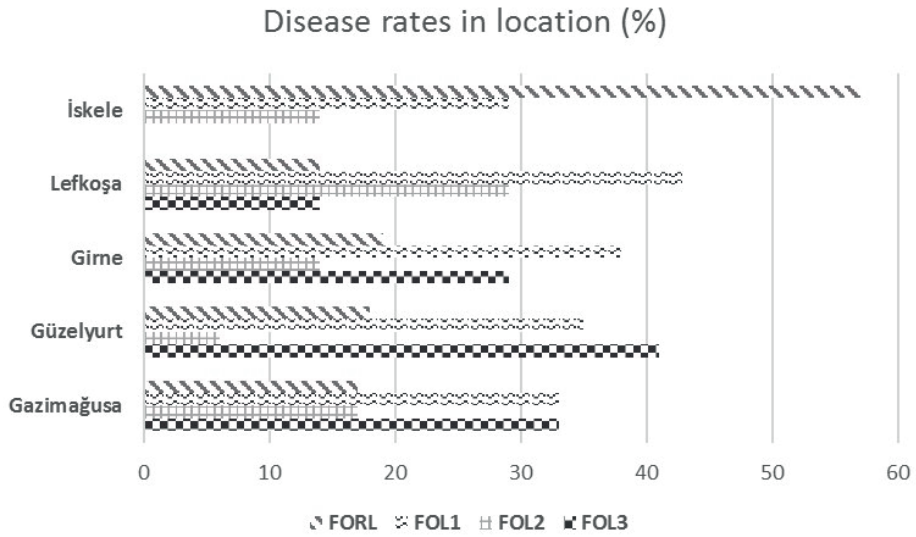

Fig. 1. FOL and FORL disease rates in tomato cultivation areas

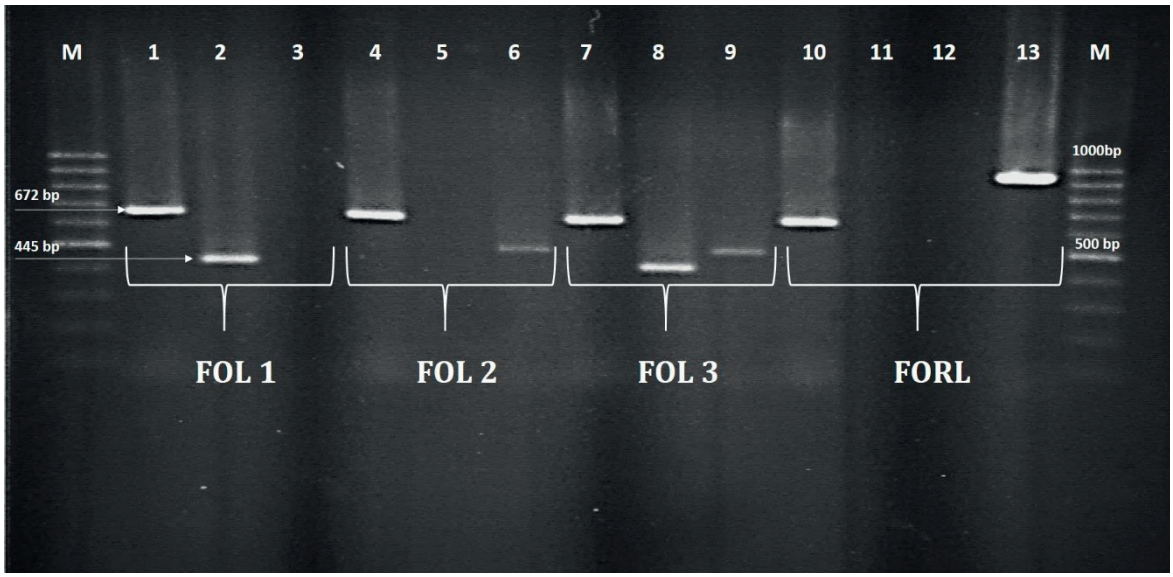

Fig. 2. Agarose gel image of specific PCR products obtained from *Fusarium oxysporum* isolates. M: 100 bp molecular marker, 1, 4, 7, 10: uni primer *Fusarium oxysporum*; 2, 8: sp 13 primer 445 bp; 6, 9: sp 23 primer 518 bp; 13: sprl primer 947 bp

challenging to control these pathogens [Katan et al. 1975, Çolak 2018]. In the same areas, it was also noticed that crop rotation application was not carefully implemented for tomato cultivation. The spread of *Fusarium oxysporum* formae speciales and its strains in the region did get proper attention until the harvesting. Cleaning the greenhouse soil's beds and improper disposal of the harvested residues are able to commence outbreaks inside the greenhouse. These diseases were frequently observed in other greenhouses that are adjacent to the greenhouses that dispose of the harvest residues around the greenhouses and in irrigation canals. The resistant spore formed chlamydospore that can survive for many years in soil and plant waste,

the chlamydospor considered to be a problem in tomato cultivation areas and cause significant economic damage [Jones et al. 1991]. In this respect, it is concluded that regional tomato producers have inadequate knowledge (crop rotation, proper fertilization, proper soil pH, destruction of greenhouse and surrounding plant residues, soil disinfection) on the control of soilborne pathogens, 76 samples from plants with disease symptoms were investigates for TYLCV infection. Based on the PCR study conducted with strain-specific primers, *Tomato yellow leaf curl virus* – Israel (TYLCV-Is) 634 bp, *Tomato yellow leaf curl virus* – Sicilia (TYLCV-Sic) 946bp, *Tomato yellow leaf curl virus* – Mild (TYLCV-Mld) 316 bp, *Tomato yellow leaf curl virus* – Sardinia (TYLCSV-Sa) were determined in infected plants (Fig. 3). Once the TYLCV virus was symptomatologically examined, it was not possible to discern the strains from the symptoms in plants. Therefore, molecular methods were considered as the most effective and reliable way in diagnosing of the disease [Fauquet et al. 2005, Anfoka et al. 2008].

While TYLCV-Is, TYLCV-Mld, and TYLCV-Sic strains were detected in two different Multiplex PCR combinations, the TYLCV-Sar strain was not observed in NC tomato cultivation areas. These identified strains were the most common TYLCV strains in the Mediterranean basin [EPPO 2005.] As a result of PCR analyses, TYLCV-Is strain was detected as a single or mixed infection in 82% of the 62 of the 76 collected samples. For TYLCV-Mld and TYLCV-Sic these values were 33% and 9,12% respectively (Tab. 7). Furthermore, mixed infection of TYLCV-Is + TYLCV-Mld strains was detected in 23 samples (37%) and TYLCV-Is + TYLCV-Sic in 5 samples. Although previous studies identified that the TYLCV strain was highly frequent in subtropical areas, where whitefly caused almost a threshold for economic loss, no prior studies existed for the tomato plant cultivation in NC, thus a comparison was not available [Fidan et al. 2019, Anfoka et al. 2008, Czosnek 2008]. In line with these studies, the present study provided an exact and reliable diagnosis of the single or mixed infection of the tomato cultivation in NC with TYLCV with Israel, Sardinia and Mild strains through the use of molecular techniques.

The facts that Cyprus is an island, a significant stop in sea transportation and its plant diversity are considered effective in the spread of diseases. The presence of TYLCV in cultivation areas is not known and there-

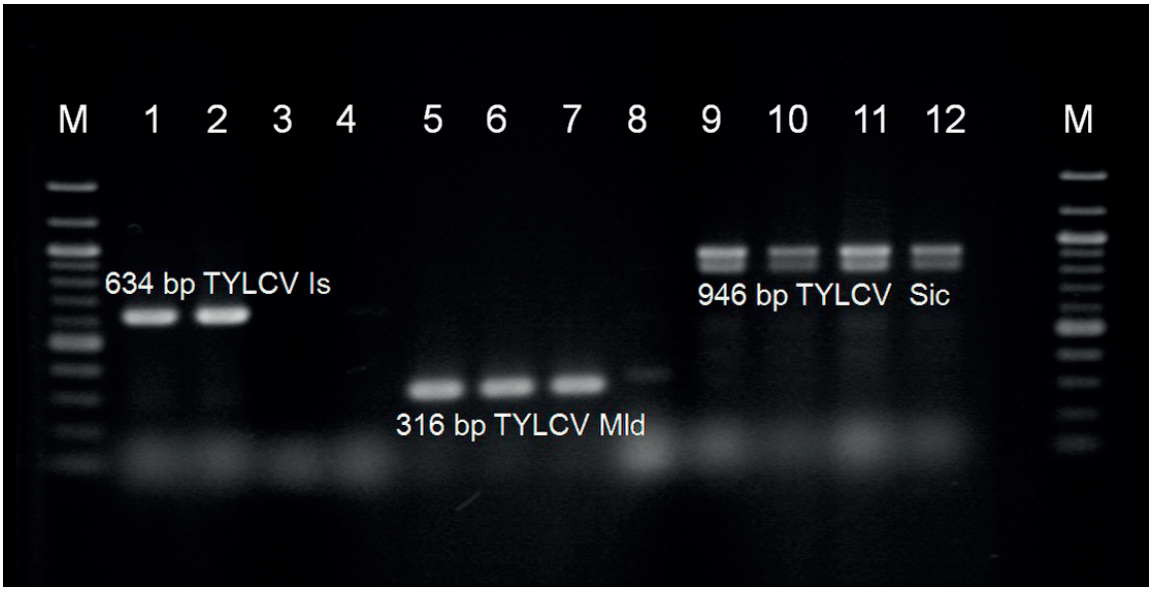

Fig. 3. Agarose gel image of PCR results with primers specific to TYLCV strains. M: 100 bp DNA ladder 1, 2; *Tomato yellow leaf curl virus*-Israel (TYLCV-Is) 634 bp 3, 4; negative control 5, 6, 7, 8; *Tomato yellow leaf curl virus*-Mild (TYLCV-Mld) 316 bp 9, 10, 11, 12; *Tomato yellow leaf curl virus*-Sicilia (TYLCV-Sic) 946 bp

Table 7. Detection of TYLCV strains with Multiplex PCR sets

Location	Infected plant number	TYLCV-Is	TYLCV-Mld	TYLCV-Sic	TYLCV-Sa	TYLCV-Is + TYLCV-Mld	TYLCV-Is + TYLCV-Sic
Gazimağusa	19	16	6	4	0	7	1
Güzelyurt	20	17	7	2	0	4	2
Girne	14	11	3	2	0	5	-
Lefkoşa	5	4	2	1	0	3	-
İskele	18	14	7	3	0	6	2
Total	76	62	25	12	0	23	5

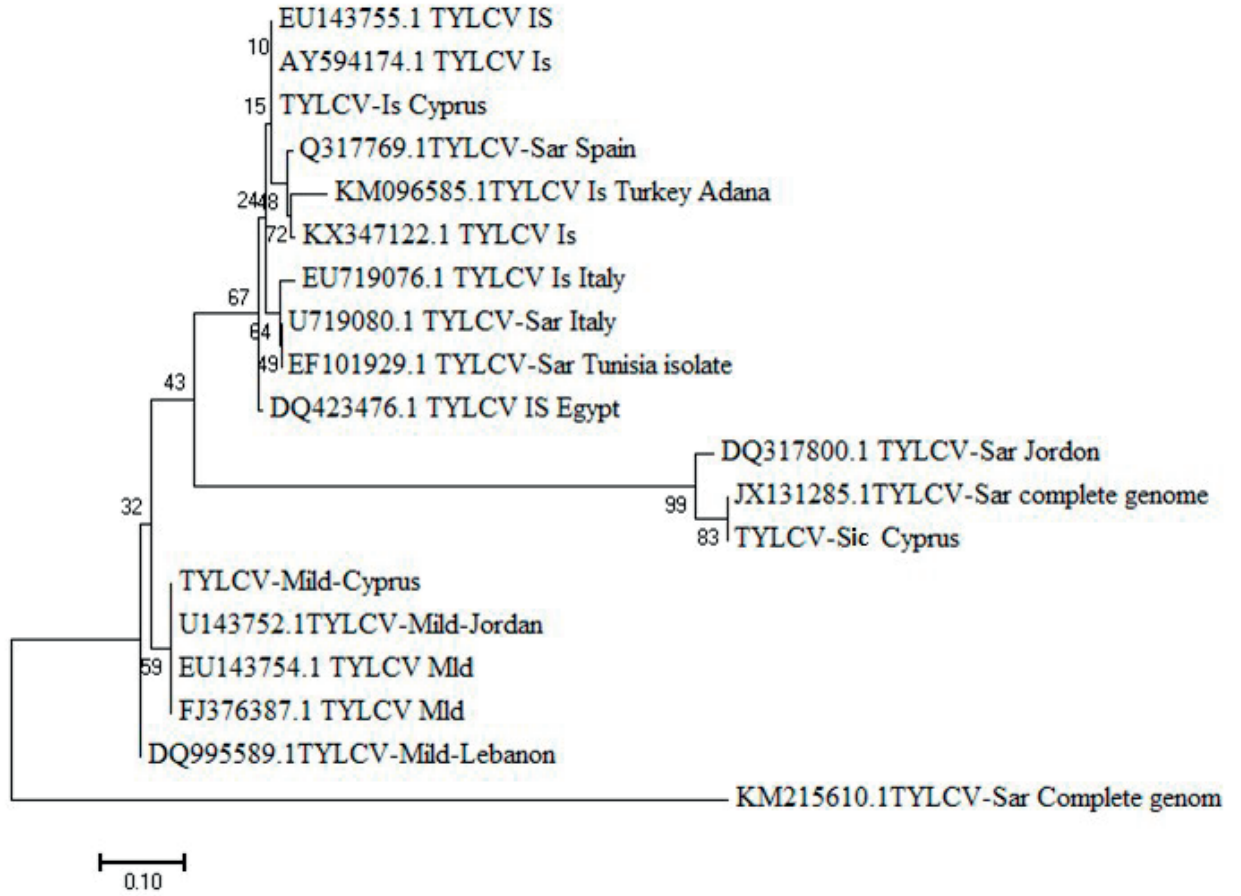

Fig. 4. The phylogenetic tree created using the MEGA 7 program according to the nucleotide similarity index of TYLCV strains in NC tomato production areas

fore it becomes impossible to correctly diagnose and to use resistant species, thus the TYLCV infection rates increase. Furthermore, the whitefly population is high on the island and they help the spread the infection between the regions. The high rate of infection in Güzelyurt, Gazimağusa and İskele regions, where tomato cultivation is done intensively, occurs due to uncontrolled seedling trade and high whitefly population. It is acknowledged that the rapid spread of TYLCV infection is inevitable in areas where the whitefly population is high and TYLCVs exist [Bel-Kadhi 2008, Ghanim et al. 1998]. In such areas, it is important to use resistant species and to effectively combat with whitefly populations [Anfoka et al. 2008, Fidan et al. 2011].

Direct sequencing service was received for the obtained PCR products and the nucleotide sequences were acquired. The comparison of the sequences with world isolates was conducted via the NCBI (National Center for Biotechnology Information) (Fig. 4). The comparisons revealed that TYLCV- Israel isolates was similar to from Lebanon, Israel and Morocco isolates from 97 in 98% and Mild isolates exhibited 96 to 99% similarity with the isolates from Lebanon Spain, Italy, and the US. Sicilian isolates, on the other hand, exhibited 94 to 97% similarity with the isolates from Italy, Spain and Morocco (EU143755.1, AF260331.1, FJ012359.1, EU719081.1). Haplotype analyses were evaluated in dnasp5.exe software. Once the isolates collected from the five regions of NC, where tomato cultivation is carried out, were compared to each other it was found that the TYLCV-Is isolates had a single haplotype and each of the TYLCV-Sic and TYLCV-Mld isolates were found to have different haplotypes.

The phylogenetic analysis utilizes that the tree is separated into two main groups (Group I and Group II) and Group I divides to 3 subgroups. TYLCV-Mild isolate from Cyprus has a unique haplotype which is clustered in Subgroup 1C. TYLCV Is Cyprus isolates from Egypt, Italy, Spain, and Turkey Group 1A alongside with TYLCV-Is isolate. and TYLCV-Sic isolates are clustered in Group 1B with other Jordan and Spain isolates which shows that these isolates are related in the terms of phylogenetic (Fig. 4), In the phylogenetic tree created with one isolate belonging to each strain demonstrated that these isolates shared the same origin with the Mediterranean countries, especially the TYLCV- Israel strains shared the same origin with Turkey, Israel, Jordan, Italy, and Egypt, therefore, being an island in the middle of Mediterranean, Cyprus is in interaction in terms of the diseases that spread due to maritime trade. Based on the PCR results of the strain-specific primers of the 62 of 76 collected samples, the most common strain was determined as TYLCV-Israel with 82%. TYLCV-Israel is followed by TYLCV-Mld with 33% and TYLCV-Sic with 16%. In addition, mixed infection of TYLCV-Is + TYLCV-Mld was detected in 23 samples with a ratio of 30% (Tab. 7). Mixed infection of TYLCV-Is + TYLCV-Sic was also detected in 5 samples [Abak et al. 1991, Ghanim et al. 1998, Accotto et al. 2000, Moriones and Navas-Castillo 2000, Anfoka et al. 2008, Fidan et al. 2011].

CONCLUSION

In the present study, the symptomatologic and molecular diagnosis of the *Fusarium* species and *Tomato yellow leaf curl virus* which cause paleness and root and collar rots in plants grow greenhouse and field cultivation at different regions of NC were identified and their prevalence was investigated. The outcomes of the present study are expected to encourage for development of resistant tomato species to the *Fusarium oxysporum* formae speciales and strains *Tomato yellow leaf curl virus*. Both pathogens cause significant yield losses in tomato grown areas. Consequently, it would be more appropriate for the producers to use and develop tomato species that are resistant to the form and the strain of the disease determined in the region. Currently, breeding programs commonly focus on developing species that are resistant to the diseases. Since this approach is the most effective and economic method for pest controlling process of plant diseases.In order to increase efficiency in the fight against soil borne pathogens, as well as a selection of resistant varieties, it should be considered as a whole system for the protection of environmental resistance. To control with soil-borne pathogens should be done in the absence of the plant before planting. In this context, in order to ensure a long-term and effective controlin soil-borne diseases, it is important to take crop rotation, the use of resistant varieties, solarization (soil disinfection) and all cultural measures together [Koike et al. 2003, Çolak et al. 2018].

TYLCV virus exists in these regions and there is no pest-control strategies via the use of chemicals. The TYLCV is one of the most important virus diseases in the tomato cultivation in NC. The present research determined that TYLCV-Israel was the most common strain in the island, then Mild and Sicilia strains followed it. In order to prevent the entrance of new virus strains to the island, we should use proper quarantine applications and pest-control for the present strains. It is necessary to use resistant species and to apply integrated pest-control methods towards vectors such as whiteflies play an important role in the spread of these virus diseases. In conclusion, it is essential to conduct health checks on the plant materials at the ports and to use effectively internal and external quarantine in order to eliminate these diseases.

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