Plant Archives Volume 20 No. 2, 2020 pp. 4511-4514 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR IDENTIFICATION OF SOME PHYTOPLASMA ISOLATES COLLECTED FROM SOME ECONOMIC CROPS AND WEEDS IN MIDDLE EUPHRATES REGION OF IRAQ

Maytham Bahar[1], Fadhal A. Al-Fadhal[1]* and Aqeel N. AL-Abedy[2]

[1]Plant Protection Department, Faculty of Agriculture, University of Kufa, Iraq.
[2]Plant Protection Department, College of Agriculture, University of Kerbala, Iraq.

Abstract

This study was conducted to isolate and identify seven phytoplasma isolates from different plant samples collected during the growing season of 2018-2019 from some fields situated in some governorates (Najaf, Karbala, Qadisiyah, Babylon and Basra) in Iraq. PCR amplification and DNA sequencing showed that five phytoplasma isolates (1, 2, 3, 4, 5) were found to be previously recorded in NCBI. Whereas, the other two isolates (6 and 7) showed a variation (96%-99%) in the nucleotide sequences with other phytoplasma isolates deposited in NCBI.

Key words: molecular identification, phytoplasma, PCR, DNA sequencing, genetic similarity, Neighboring-joining tree.

Introduction

Phytoplasmas are plant pathogenic bacteria in the class Mollicutes and are formally called mycoplasma-like organisms (MLOs) (Doi *et al.,* 1967). They are transmitted by insect vectors (leafhoppers, planthoppers and psyllids) and infect hundreds of plant species worldwide, including many economically important crops, fruit trees and ornamental plants (Hogenhout *et al*., 2008; Oshima *et al*., 2013). Infected plants show a wide range of symptoms including stunting, yellowing, witches' broom (development of numerous tiny shoot branches with small leaves), phyllody (formation of leaf-like tissues instead of flowers), virescence (greening of floral organs), proliferation (growth of shoots from floral organs), purple top (reddening of leaves and stems) and phloem necrosis. The diseases caused by these Phytoplasmas can significantly reduce the yield like purple top roll, marginal flavescence and witches broom causing yield reduction by up to 50-75%, 50-95% and 15%-65%, respectively (Nagaich *et al.,* 1982).

The ability to detect and identify phytoplasmas is necessary for accurate disease diagnosis. However, detection has been hampered by the inability to culture these prokaryotes *in vitro*. Therefore, alternative methods must be used to detect and characterize phytoplasmas. Polymerase chain reaction (PCR) has since proven to be a more versatile tool for detecting phytoplasmas in their plans and insect hosts (Deng *et al.,* 1991; Ahrens *et al.,* 1992; Lee *et al.,* 1993).

The objective of this study was to isolate and identify some isolates of phytoplasma, collected from different plants grown in some governorates in Iraq, using the polymerase chain reaction (PCR) as well as to determine the nucleotide sequences of the PCR-amplified products to investigate the relationship between these isolates identified in this study and with the other isolates registered in GenBank database.

***Author for correspondence :** E-mail: fadhl.alfadhl@uokufa.edu.iq

Materials and Methods

Sources of phytoplasma isolates and DNA extraction

Seven samples (1-tomato (*Solanum lycopersicum* L.), 2-eggplant (*Solanum melongena* L.) from Najaf and 3-sesame (*Sesamum indicum* L.), 4-Suaeda (*Schanginia baccata* MQ.T), 5-wild basil (*Clino podium*), 6-tomato (*Solanum lycopersicum* L.), 7-Arabian jasmine (*Jasminum sambac*) from Basra were collected during the growing season of 2018-2019 from some fields located in some provinces (Najaf and Basra) in Iraq. These samples were collected from some plants showing symptoms of stunting, virescence, shortened internodes, big bud, little leaf, witches broom, phyllody,

Table 1: Similarity rates among seven Phytoplasma isolates diagnosed in this study.

Isolate no.							
1	-						
2	98	-					
3	98	98	-				
4	98	98	100	-			
5	98	98	100	100	-		
6	98	98	100	100	100	-	
7	98	98	100	100	100	100	-
	1	**2**	**3**	**4**	**5**	**6**	**7**

giant calyx and floral malformation. Totao DNA extraction was performed using the Geneaid Plant Mini Kit® (Cat. No: GP100) following the manufacturer's instructions. The quality and quantity of DNA extracted from each infected sample were measuredusing a UV spectrophotometer and then DNA samples were kept at -20°C until use.

PCR amplification and DNA sequencing

PCR amplification was done using the Maxime PCR Premix (i-Taq) kit (Cat. No. 25026) with the primer set (F; 5'-ACGAAAGCGTGGGGAGCAAA-3') and (R: 5' GAAGTCGAGTTGCAGACTTC 3') to amplify a 500bp product from each sample (Ahrens and Seemüller, 1992). The targeted region of Phytoplasma genome was amplified from all samples using the following PCR conditions: Initial DNA denaturation for 5 minutes at 94°C, followed by 35 cycles each consisting of final denaturation for 52 seconds at 94°C, primer annealing for 40 seconds at 52°C and thereafter an initial extension for 1 min at 72°C with completing all CR steps by one cycle of the final extension at 72°C for 5 min. PCR-amplified products were separated on a 1% agarose gel for 150 min and examined using ethidium bromide staining under UV illumination and gel pictures were taken.

For DNA sequencing, the purified PCR products were sent along with the primer pair to Microgen biotechnology company (South Korea). To diagnose the Phytoplasma isolates and to determine the level of similarity and difference in the obtained nucleotide sequence, the sequence of each Phytoplasma isolate was searched in NCBI using Basic Local Alignment Search Tool (BLAST) software (Zheng *et al.*, 2000). The nucleotide sequences were used to construct the phylogenetic tree using MEGA6 software, using the Neighbor-joining method (Tamura *et al.*, 2013).

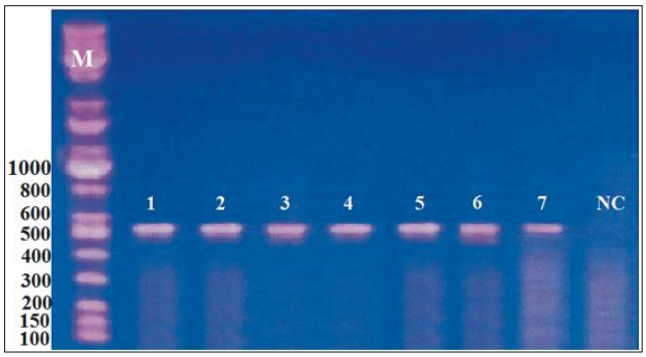

Fig. 1: PCR products amplified from the Phytoplasma isolates 1, 2, 3, 4, 5, 6 and 7 isolated in this study from some plants.

Results

Molecular identification of different Phytoplasma isolates

A PCR product of approximately 500bp was amplified from each isolate collected isolated from different plants in Najaf, Karbala, Qadisiyah, Babylon and Basra provinces (Fig. 1). Analysis of nucleotide sequences generated from each PCR-amplified product was done using BLAST search at NCBI database. This analysis showed that all the identified isolates belong to Phytoplasma.

Alignment of nucleotide sequences of the Phytoplasma isolates 1 and 2 with the nucleotide sequences of the other isolates 3, 4, 5, 6 and 7 showed a similarity of 98% (Fig. 2, Table 1). It was also revealed that the nucleotide sequence of Phytoplasma isolates 3, 4, 5, 6 and 7 had a similarity of 100% (Table 1). However, the nucleotide sequences of the Phytoplasma isolates 3, 4, 5, 6 and 7 were found to be previously reported and registered in NCBI with a similarity of 100%. The phylogenetic tree constructed using the nucleotide sequences of the identified Phytoplasma showed that the

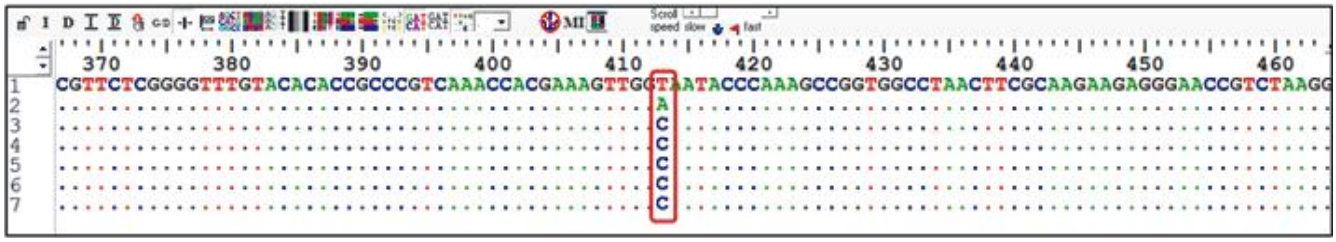

Fig. 2: Alignment sequences shows the similarities and differences in the nucleotide sequences of the Phytoplasma isolates 1, 2, 3, 4, 5, 6 and 7 diagnosed in this study. Identical nucleotides are shown in dots. Numbers on the right side of the figure represent nucleotide sequences of the investigated hytoplasma isolates.

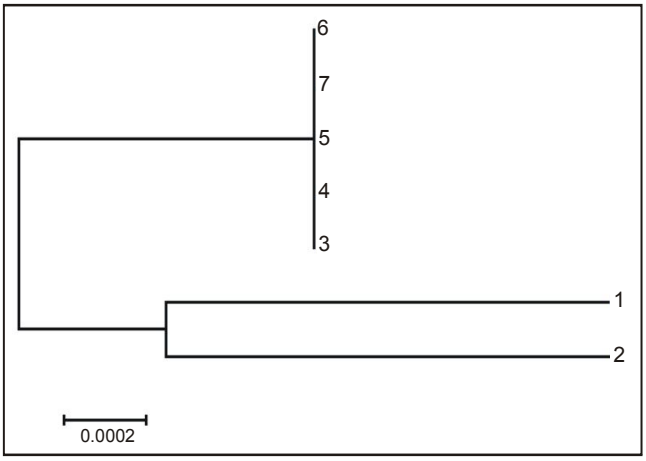

Fig. 3: Neighboring-joining tree states the genetic relationship for the Phytoplasma isolates investigated in this study.

isolates 1 and 2 appeared in a separate clade apart from the other isolates 3, 4, 5, 6 and 7 (Fig. 3).

The BLAST search of the Phytoplasma isolate 1showed a clear difference (98%) in the nucleotide sequence with the nearest nucleotide sequence of the Phytoplasma isolate (CP055264.1) previously deposited at NCBI (Fig. 4). Besides, this isolate gave a nucleotide sequence similarity ranging from 96-98% with the other phytoplasma isolates previously recorded at NCBI. It was also found from the neighbor-joining tree that Phytoplasma isolate 2 was appeared in a clade separated from the other isolates with a similarity percentage ranged between 96-99% with other Phytoplasma isolates recorded in NCBI (Fig. 5).

In this study, seven Phytoplasma (1, 2, 3, 4, 5, 6 and 7) were isolated from different plants and identified using the PCR technique. PCR identification was efficiently used in identifying many microorganisms including fungi, bacteria, viruses and Phytoplasma (Kazmi *et al.,* 2007; Iftikhar *et al*., 2011; AL-Abedy, 2018; Al-Fadhal *et al*., 2018; Al-Fadhal *et al*., 2019).

Conclusions

• A PCR product of approximately 500bp was amplified from each isolate collected isolated from different plants in Najaf and Basra provinces. Analysis of nucleotide sequences generated from each PCR-

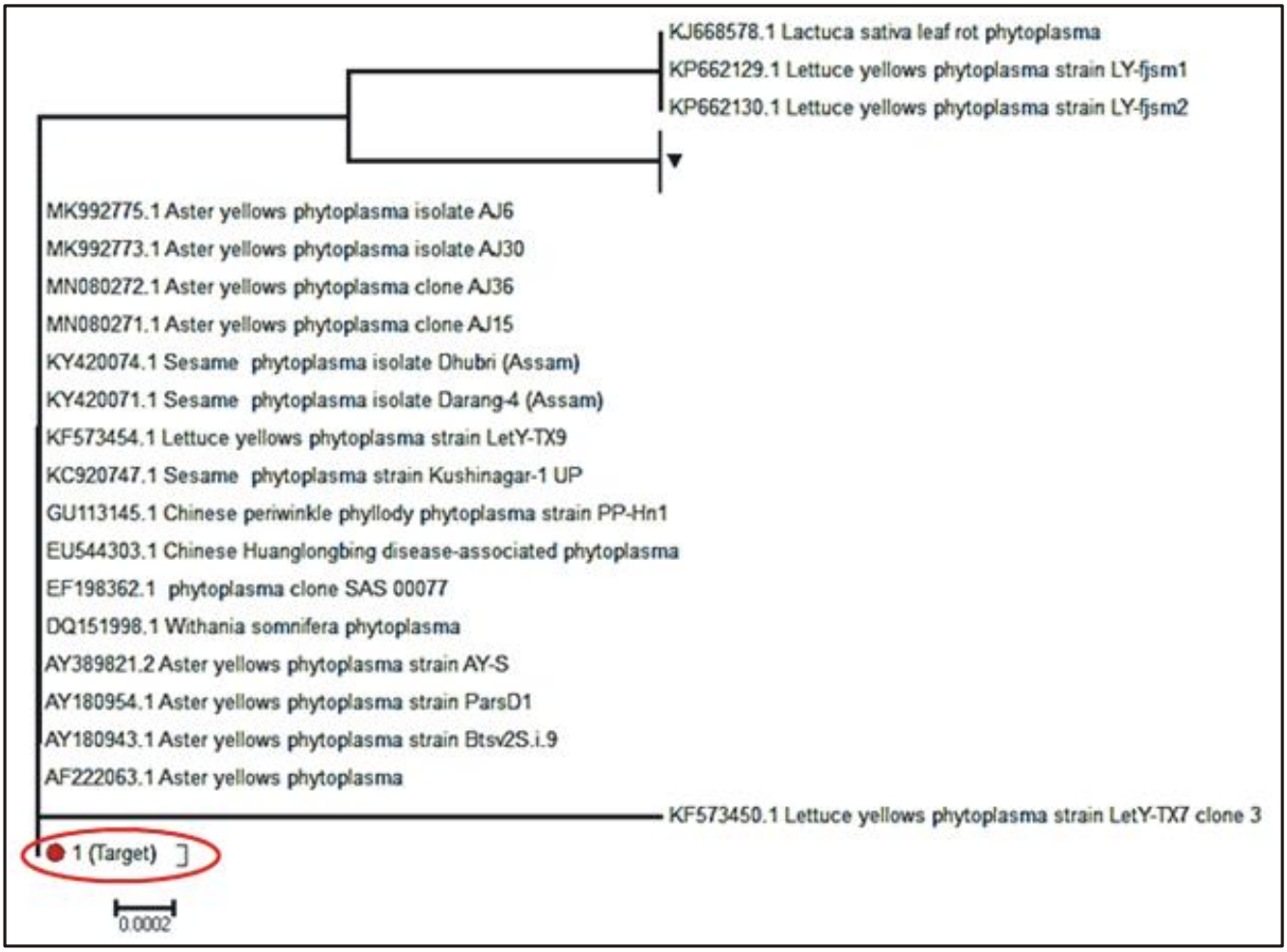

Fig. 4: Neighboring-joining tree shows the relationship for the Phytoplasma isolate (1) investigated in this study and the other isolatesformerlyrecorded at NCBI.

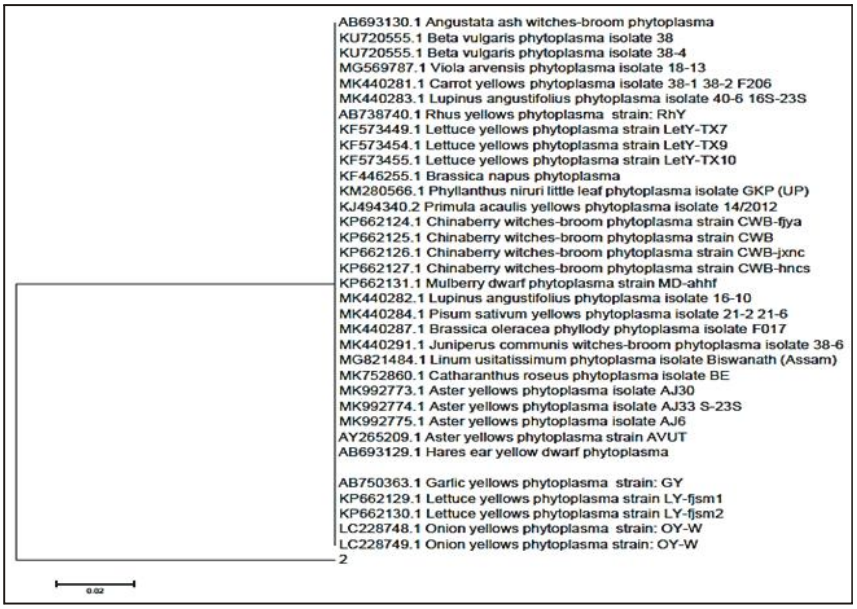

Fig. 5: Neighboring-joining treeshows the relationship for the Phytoplasma isolate (2) investigated in this study and the other isolated previously registered at NCBI.

amplified product using BLAST search at NCBI database showed that all the identified isolates belong to Phytoplasma.

• Alignment of nucleotide sequences of the Phytoplasma isolates 1 and 2 with the nucleotide sequences of the other isolates 3, 4, 5, 6 and 7 showed a similarity of 98%. It was also revealed that the nucleotide sequence of Phytoplasma isolates 3, 4, 5, 6 and 7 had a similarity of 100%. However, the nucleotide sequences of the Phytoplasma isolates 3, 4, 5, 6 and 7 were found to be previously reported and registered in NCBI with a similarity of 100%. The phylogenetic tree constructed using the nucleotide sequences of the identified Phytoplasma showed that the isolates 1 and 2 appeared in a separate clade apart from the other isolates 3, 4, 5, 6 and 7.

References

Ahrens, U. and E. Seemüller (1992). Detection of DNA of plant pathogenic mycoplasmalike organisms by a polymerase chain reaction that amplifies a sequence of the 16 S rRNA gene. *Phytopathology*, **82(8):** 828-832.

AL-Abedy, A.N., F.A. Al-Fadhal, M.H. Karem, Z. Al-Masoudi and S.A. AL-Mamoori (2018). Genetic variability of different isolates of *Rhizoctoniasolani*Kühn isolated from Iranian imported potato tubers (*Solanumtuberosum* L.). *Int. J. Agricult. Stat. Sci*., **14(2):** 587-598.

Al-Fadhal, F.A., A.N. AL-Abedy and D.A. Alkhafije (2019). Isolation and molecular identification of *Rhizoctoniasolani* and *Fusariumsolani* isolated from cucumber (*Cucumissativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillussubtilis*. *Egyptian Journal of Biological Pest Control*, **29(1):** 47.

Al-Fadhal, F.A., A.N. AL-Abedy and M.M. Al-Janabi (2018). Molecular identification of novel isolates of RhizoctoniasolaniKühn and *Fusarium* spp. (Matsushima) isolated from petunia plants (*Petunia hybrida* L.). *Plant Archives,* **18(1):** 703-711.

Deng, S. and C. Hiruki (1991). Amplification of 16S rRNA genes from culturable and nonculturablemollicutes. *Journal of microbiological methods*, **14(1):** 53-61.

Doi, Y.O.J.I., M. Teranaka, K. Yora and H. Asuyama (1967). Mycoplasma-or PLT group-like microorganisms found in the phloem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellows, or paulownia witches' broom. *Japanese Journal of Phytopathology*, **33(4):** 259-266.

Hogenhout, S.A., K. Oshima, E.D. Ammar, S. Kakizawa, H.N. Kingdom and S. Namba (2008). Phytoplasmas: bacteria that manipulate plants and insects. *Molecular plant pathology*, **9(4):** 403-423.

Iftikhar, S. and F.A.U.Q.I.A. Fahmeed (2011). Detection of phytoplasma from diseased potato samples. *Pakistan J. Botany*, **43:** 1085-1090.

Kazmi, M.R., S.I. Farooq, F.S. Fateh, S.H.A.H.I.D. Hameed, F.A.U.Q.I.A. Fahmeed, M.U.H.A.M.M.A.D. Ashraf and I. Ahmed (2007). Identification of phytoplasma from mango trees showing sudden death phenomenon through molecular techniques. *Pak. J. Bot.*, **39(6):** 2153-2157.

Lee, I.M., R.W. Hammond, R.E. Davis and D.E. Gundersen (1993). Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. *Phytopathology*, **83(8):** 834-842.

Nagaich, B.B., S.M.P. Khurana, S. Sing, V. Sunaina and S.B.S. Parihar (1982). Studies on the transmission and control of Potato yellow virus and their effect on crop production. Potato in Dev. Countries. Indian Pot. Assco., Simla. 294-03.

Oshima, K., K. Maejima and S. Namba (2013). Genomic and evolutionary aspects of phytoplasmas. *Frontiers in microbiology*, **4:** 230.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar (2013). MEGA6: molecularevolutionary genetics analysis version 6.0. *Mol. Biol. Evol*., **30:** 2725-2729.

Zhang, Z., S. Schwartz, L. Wagner and W. Miller (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational biology*, **7(1-2):** 203-214.