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

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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

Phytoplastasms 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 Phytoplastasms can significantly reduce the yield like purple top roll, marginal flavesence 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 phytoplastasms 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.

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 (Clinopodium), 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,
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 measured using 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).

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

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

**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 phytoplasma isolates.

**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
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 1 showed 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-
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 NCB1 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


