



University of Zabol

Graduate Management

School of Agriculture

Plant Protection Group

Thesis for obtaining a doctorate degree in plant pathology

**Detection and identification of Phytoplasmas on grapevine,
pomegranate, apricot and apple in Khorasan Razavi
province**

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Abstract

Phytoplasmic pathogens of plants are a special group of cell wall-free prokaryotes that are found only in the phloem tissues of plants and their cultivation in microbiological environments is difficult or almost impossible. In recent years, these symptoms have been observed in some provinces of Khorasan Razavi, and every year it has caused significant damage to these orchards. The aim of this study was to detect and identify different groups of phytoplasma in infected orchards. During spring and summer of 2018, suspected symptoms of the phytoplasma disease such as leaf shrinkage and yellowing as well as leaves with abnormally-enlarged stipules were observed in grapevine, pomegranate, apricot and apple orchards in Khorasan Razavi province and samples were collected from leaves and petioles. Samples of symptomatic tree were assayed for infection with phytoplasma from extracted DNA using universal primer pairs P1/P7 and nested PCR using primer pair R16F2n/R16R2, fU5/rU3 and R16MF2/R16MR2 in Polymerase Chain Reaction (PCR). The universal PCR products were 1800 bp fragments and the nested-PCR products were 1250, 876 and 1400 bp fragments, respectively, which were amplified by primer pairs. The R16MF2/R16MR2 primed nested PCR products were cloned, sequenced and registered to the Genbank. Comparison of the obtained sequences with the sequences of NCBI using BLAST software and Restriction Fragment Length Polymorphism (RFLP) analysis of the nested PCR products using the enzymes *AluI*, *RsaI* and *TruI* (*MseI*) indicated that phytoplasma in grapevine and pomegranate trees was most similar to *Candidatus* Phytoplasma trifolii related to Clover proliferation group (16SrVI), and *Candidatus* Phytoplasma solani related to Stolbur group (16SrXII). Moreover, in apricot and apple trees phytoplasma was most similar to *Candidatus* Phytoplasma trifolii related to Clover Proliferation (CP) 16SrVI group. Analysis of sequences using iPhyClassifier software, determination of nucleotide similarity, and phylogenetic analysis of full-length 16S rDNA showed that phytoplasma in grapevine, pomegranate, apricot and apple trees of Razavi Khorasan province belongs to 16SrVI-A and 16SrXII-A subgroup. This is the first report on *Ca. Phytoplasma trifolii* infections in apple, pomegranate and apricot trees and *Ca. P. solani* (16SrXII) in pomegranate trees in Iran and other regions across the world.

Keywords: Nested PCR, Phytoplasma, RFLP