

University of Zabol

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The Thesis Submitted for the Degree of M.Sc (in the field of Plant Pathology)

Identification of phytoplasmas associated with witche's broom diseases of Khafr almond and Estahban GF-677 based on biological and molecular characteristics

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Abstract

GF-677 rootstock hybrid (Prunus amygdalus x Prunus persica) having a strong roots, is a good rootstock with high potential for pests and diseases controlling. GF-677 cuttings have been imported from Europe to Iran and planted in some of areas in Fars province for further propagation and distribution to other Iranian stone fruit growing zones. Previous studies have been demonstrated that, phytoplasma agent of GF-677 witches'- broom belong to 16SrIX phytoplasma group. In the present study, phytoplasmal agents of Almond witches'-broom from Khafr strain (KAWB) and GF-677 witches'broom from Estahban strain (EGWB), were compared using partial biological and molecular characteristics. Phytoplasmal agents of KAWB and EGWB diseases were transmitted to periwinkle plants via dodder and grafting method. Base on our observation any phytoplasma typical symptoms as virescence and phyllody was not showed in periwinkle infected by KAWB phytoplasmal agent whereas EGWB phytoplasma agent were caused these symptoms in periwinkle. Total genomic DNA from midribs tissue of fresh leaf of periwinkle plants infected by both phytoplasma strains were extracted. A 1800 and 350 fragment of in ribosomal RNA operon were amplified using P1/P7 and P3/P7, respectively. Nested R16F2/ R16R2 pair primer was used to amplify a 1200 bp fragment from PCR products in previous step. Another direct PCR using universal rpF1/rpR1 primer pair was confirmed to amplify a phytoplasma DNA segment (1245-1389 bp) of the ribosomal protein operon. Based on the results of BLAST search against sequenced products of PCR using P3/P7 and rpF1/rpR1 both phytoplasmas were placed in the PPWB (16SrIX) group but virtual RFLP patterns obtained from restricted fragment by DraI 'HhaI 'RsaI and TaqI restriction enzymes revealed that this two phytoplasmas are different and belonging 16SrIX-C and 16SrIX-B subgroup, respectively. In physichal RFLP, comparison of digestion patterns from direct PCR products amplified by P1/P7 primer pair using DraI and MboI restriction enzymes and also rpF1/rpR1 primer pair using HaeIII and DraI restriction enzymes revealed that KAWB and EGWB agents are not closely related. Phylogenetic analyses and percent sequence homology of ribosomal RNA and ribosomal protein revealed that KAWB and EGWB phytoplasmas are clearly different from each other and EGWB is classified with candidatus phytoplasma phoenicium (16SrIX-B) in Iran (Neyriz) and Lebanon.

Key words: Almond, Phytoplasma, Witche's broom, Molecular analysis