

PARTIAL CHARACTERIZATION OF A PHYTOPLASMA ASSOCIATED WITH ALFALFA WITCHES' BROOM IN BUSHEHR PROVINCE*

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Abstract

Alfalfa witches' broom (AWB) is a serious disease of alfalfa in Bushehr province. For characterization of Bushehr AWB phytoplasma, alfalfa plants with typical symptoms of AWB were selected in different regions of Bushehr province including Bondarooz(1 isolate), Sarkoreh (2 isolates), Poshtpar(1 isolate) and Tange- Eram (1 isolate), transferred to greenhouse and used as the source of the disease agent for transmission and molecular studies. Agent of AWB was transmitted from each isolate to periwinkle via dodder inoculation. Agents of all isolates caused little leaf, internode shortening, yellowing, virescence, phyllody and witches' broom symptoms in periwinkle plants. DNA fragments of approximately 1800 bp of ribosomal operon were amplified with primer pair P1/P7 by direct PCR from total DNA samples extracted from naturally alfalfa infected plants and periwinkle plants inoculated with agents of AWB isolates. P1/P7 primed PCR products (1800 bp) were analyzed by digestion with *AluI*, *HpaII*, *CfoI*, *HaeIII* and *RsaI* enzymes. RFLP patterns analyzed with these enzymes were similar to each other and those of AWB phytoplasma from Fars (Mobarak Abad and Juyom 1) and Yazd, 16SrII group Related phytoplasmas but distinguishable from a Fars isolate of AWB(Juyom2) by a *CfoI* site. Phylogenetic analyses of full length 16S rRNA gene sequence also clustered agent of Bondarooz AWB phytoplasma with phytoplasmas of 16SrII group. Based on the results of the present study, a phytoplasma related to peanut witches' broom (16srII) is associated with AWB disease in Bushehr province. This is the first report of AWB in Bushehr province

Keywords: Alfalfa, Witches' broom, PCR, RFLP, Phylogenetic analysis, Bushehr province

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