University of Zabol
Graduate school
Faculty of Agriculture
Department of Plant Protection

The Thesis Submitted for the Degree of M. Sc
in the field of Plant Protection

Title:

Genetic Diversity of Hop Stunt Viroid in Affected Orange Varieties by Cachexia Disease in Mazandaran Province

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Feb 2014

Abstract

world as the agent causing cachexia disease of Citrus. Symptoms on Citrus include overall chlorosis Hop Stunt Viroid is among the most damaging viroid in the genus Hostuvirodae, most known around the of the trees, gum impregnation of the bark, depression pit in the xylem and formation of pegs on the cambial surface of the bark which fit in the pits on the wood. The severity of symptoms varies with the species cultivar of the host and strain of the viroids to estimate the prevalence of the disease in some symptomatic and symptomless species of citrus commentary grown in Mazandaran province, samples were taken randomly from sweet orange trees(Valencia, Washington navel and Blood oranges). RNA was extracted from the bark of one year old stems using TRIZOL (invitroge, Carlsbade. CA). Reverse transcriptase (RT) polymerase chain reaction (PCR) was performed using specifics primers for hop stunt viroid and the product were electrophoresed on 2% agarose gels. The expected fragments of 300bp typical of hop stunt viroid, were detection in 40/12% samples. The HSVd DNA bands were purified using High Pure PCR Product Purification Kit (Roch, Germany) and subjected to Single-strand conformation polymorohism (SSCP) analysis. Diversity in each cultivar was compared and finally 4 different pattern was discovered. These isolates were sequenced in both direction to confirm SSCP analysis. Alignment of the nucleotide sequences by BLASTn (NCBI) program revealed marked identity with other HSVd isolates deposited in Genbank. A future study was, CEVd company with HSVd in randomly symptomatic trees which infected by Cachexia disease. PCR products were electrophoresed on 2% agarose gel and the CEVd specific bands were observed.

Key words: Genetic Diversity, Cachexia, Hop Stunt Viroid