Abstract

Wilsonomyces carpophilus, the casual agent of shot hole disease of stone fruit trees, is one of the most important diseases of stone fruits world wide The disease can weaken the trees and reduce the quantity and quality of the crops. The objective of the research was to study the genetic diversity of *W. carpophilus* in Khorasan Razavi province using two different molecular fingerprinting methods: rep-PCR and RAPD-PCR. Sampling was perform from peach, nectarine, plum, apricot and cherry orchards of Quchan, Torqabeh-Shandiz, Chenaran, Neishabur, Kalat, Torbat Heidarieh and Mashhad cities. Infected tissues including leaves, twigs, and fruit were transferred to the laboratory. After surface sterilization, three samples from each isolate were cultured on PDA, MEA, and WA media and incubated at 18, 20, and 25°C. the fungi werw purified using single spore technique.then were identified through valid resources. The research was performed on 20 fungal isolates collected from different stone fruit trees. After genomic DNA extraction, the DNA was amplified using 13 RAPD random primers, and BOX A1R, ERIC2, ERIC1R, REP2-I, and REP1R-I primers. PCR reaction with rep-PCR marker resulted to amplification of numerous fragments of DNA. Thirty-eight of 39 amplified fragments (97.5 %) were polymorphic. Molecular weight of amplified DNA fragments were between 100 and 5000 base pairs. Similarity matrix between isolates was calculated based on Jaccard Coefficient and cluster analysis and construction of dendogram was done based on UPGM using NTYSIS.PC 2.0 software. The results of rep-PCR marker analysis indicated that at 69% similarity level, isolates were divided into 12 groups or lineages. The marker geographically separated isolates of Kalat and Chenaran and to some extent isolates of plum in terms of hosting from the other isolates. This marker also separated most apricot isolates in terms of hosting from other groups. The results of RAPD marker analysis indicated that isolates at 51% similarity level were divided into 12 groups and colony lineages. This marker separated isolates of plum and apricot in terms of hosting and geography from the other isolates. The result of the study revealed high genetic diversity of the pathogen in Khorasan Razavi province. It Despite, common features of the markers such as dominancy, simplicity, and high rapidity of the function of both molecular systems, rep-PCR has more poten and was more precise in detection of genetic diversity of *W. Carpophilus* populations.

Keywords: Polymorphism, molecular marker, shot hole disease.

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

The Thesis Submitted for M.Sc Degree in Plant pathology

Investigation of genetic diversity of *Wilsonomyces carpophilus* in Khorasan Razavi using molecular markers

Supervisors:
Dr. N. Panjehkeh
Dr. M. Hajian Shahri

Advisors:
Dr. S. K. Sabbagh
Mr. M. Behzadi

By:
R. Barazandeh Aq Kariz

February
2014