Abstract

Die-back caused by *Paecilomyces variotii* is one of destructive diseases of pistachio in Kerman province. To determine genetic diversity and genome sequencing of this species,sampling was randomly performed in seven region of the Kerman province. The isolates were cultured on PDA medium and then purified by hyphal tip purification method. One hundred sixteen isolates were purified and of these, twenty eight isolates were selected for the next study. The isolates was grown on PDA medium with color variable and mostly showed in deep olive buff to dark olive buff or yellow brownish with branching density phialids. DNA extraction from fugi isolates was done by CTAB method. Genetic variation study was assayed within the Internal Transcribed Spacer (ITS) region of the ribosomal RNA gene (rDNA-ITS) using PCR-RFLP marker with one universal primer pair of eukaryotes (AB28, TW81).This primer pair amplified a fragment of 600 bp in all isolates during PCR reaction. PCR products were subjected to digestion with thirteen different restriction endonucleases enzyme as: *Eco*R I, *Hpyf* 3I, *Apa* I, *Hinf* I, *Mbo* I, *Msp* I, *Ms*e I,*Rsa* I, *Not* I, *Pst* I,*Bam*H I, *Hin*d III, *Dra* I and The digestion products were separated on a 2% agarose gel. The results showed that only seven restriction enzyme as:*Eco*R I, *Hpyf* 3I, *Apa* I, *Hin*f I, *Mbo* I, *Msp* I, *Mse* I have cut place on the used fragment. The results of enzyme digestion showed a high degree of polymorphism in different isolates. Using cluster analysis of the obtained data from the digestion reaction based on UPGMA algorithm and Jaccard's similarity coefficient, the isolates with70%similarity level were divided into 9 group. The results showed that, *Mbo* I enzyme is more appropriate enzyme than others to genetid diversity study and the isolates of Rafsanjan are more variable than that other areas. The PCR products of five isolates were selected randomly and submitted for sequencing, the obtained sequences showed an over 90% homology with sequences of other fungi existed in the gene bank. According to these finding, it has been clared that amplified fragments in the PCR reaction are related to a part of 5´regions of 18S subunit, completed region of ITS1, subunit 5.8s, ITS2 and a part of 5´ regions of 28S subunit .Alignment analysis of five selected sequencesshowed that the highest homology (98%) is present between X_3 and K_2 isolates. Also, the phylogenetic analysis and the phylogenetic tree indicated that the maximum similarity of 99% exists between X_3 and K_2 isolates. Totally, the results of phylogenetic analysis significantly confirmed the results of PCR-RFLP. The results of this research indicate that according to the fact that the isolates of an area are in the same colonial lineages but there is a specific relationship between the isolates of each area whereas no relationship was identified between distribution and variety of the isolates and the distances of geological areas of Kerman province: So we could not concludes that increasing or decreasing of distance between two areas, will increase or decrease the similarity range of the isolates. It should be noted that, although this fungi act as a opportunist agents on weak plants, furthermore, it damages extensively and the variability percentage of it outspread day to day over the province: So, according to grouping of the isolates, the controlling ways maybe vary conforming to weather conditions in different areas.

Key words: Die-back, Genetic variability, Restriction Enzyme, Sequencing, Phylogenetic Analysis

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Study of genetic variability in *Paecilomyces variotii* isolates causal agent of pistachio Dieback based on the rDNA-ITS regions using PCR-RFLP marker in Kerman province

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