

Abstract

Pistachio dieback disease is one of the most important factors in reducing the amount of pistachio production in Iran. Therefore, to investigate the genetic structure and genetic diversity during the 2013-2014 years, 400 isolates of *Paecilomyces* was isolated from pistachio orchards (branches) in Kerman, Semnan, Yazd and Khorasan Razavi provinces. With study of isolates morphological characteristics, isolates were identified as *Paecilomyces formosus*. Morphological variation was observed between isolates of *Paecilomyces*. So, based on rDNA-ITS area and beta-tubulin gene sequencing, selected strains was identified as *P. formosus* and Pistachio dieback disease in Iran. In addition, in this study, phylogenetic relationships *Paecilomyces* species were evaluated based on the sequencing of rDNA-ITS region and beta-tubulin gene. sequenced isolates of *P. formosus* have a very close relationship with *Paecilomyces lecythidis* and be formed a sister group to *Paecilomyces maximus*. In this study, it was found comparing the sequence of rDNA-ITS area and beta-tubulin gene is a way to separate the different species of the *Paecilomyces* genus. *Paecilomyces formosus* species, have high genetic and morphology diversity however; sexual stage has not been observed in pistachio orchards. In this research; the possibility of sexual stage forming in laboratory and greenhouse conditions was investigated. A primers pair including; F1Varse and R2VarMar was used for *Mat1-1* and *Mat1-2* amplification respectively by multiplex-PCR method. Mating type idiomorphs for 330 isolates of *P. formosus* belonging to pistachio trees and for 10 strains isolated from date palm trees (*Phoenix dactylifera*) were amplified. In 131 isolates (39.46%) *Mat1-1*, in 174 isolates (52.4%) *Mat1-2* and in 16 isolates (4.8%) both idiomorphs was indicated and in 11 isolates (3.3%) no band was found. The distribution of the mating types is not equal, and the natural choice has been in favor of *Mat1-2*. On the other hand, the methods used to induce sex in the lab and greenhouse conditions were not successful. In microscopic examination, no asc and ascospore were observed. The obtained results indicated that in experimental condition sexually compatible genotypes were not able to form sexual stage. Based on these results this could conclude that before genetically factors, other factor play role in sexual performance in natural condition. Genetic diversity of 100 selected isolates using SSR markers, showed a high degree of polymorphism between isolates. Cluster analysis based on UPGMA algorithm and Jaccard's similarity coefficient, divided the isolates into 10 groups with 60% similarity level. Shannon index, Principal coordinates analysis (PCoA) and analysis of molecular variance (AMOVA) revealed that the highest genetic variation (78%) was disturbed among isolates and genetic variation within isolates was determined as 22%. Between used primers, PVar15 was more appropriate than others to study of genetic diversity of isolates. Study of pathogenicity test and mean comparison mean of data showed that isolates of Kerman (kk1) and Semnan (SS1) regions had the most and lowest aggressive isolates, respectively. Our results indicated that there is a specific relationship between the isolates of each region whereas no relationship was identified between related isolates belongs to two provinces and their genetic diversity.

Key words: Morphology, Taxonomy, Phylogeny, Mating type, Population genetic, SSR



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