



Application of ISSR method in systematic identification and segregation of populations of *Aspergillus flavus*

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Introduction

Among the many species of *Aspergillus*, *Aspergillus flavus* is one of the most important species of this genus. It is one of the most common species of this genus with its high reproductive power and the multitude of different hosts, as well as the ability to produce aflatoxin (Balali et al., 2010). Identification methods based on morphological traits, although in some cases, especially in inter-species diagnosis, the methods are relatively efficient, cheap, and reliable, they are generally inefficient in distinguishing varieties and differences between *Aspergillus* varieties. In this regard, morphological traits are not considered desirable differential traits. Therefore, the use of molecular techniques to distinguish between varieties has been welcomed (Batista et al., 2008). The purpose of this research is to determine the intra-species diversity of this species in Iran and also to measure and investigate the relationship between the population diversity of this species and the climatic conditions in the country.

Materials and Method

To identify and investigate intra-species variations between *Aspergillus flavus* populations isolated from a number of widely consumed dry fruits; peanuts, dried mulberries, dried figs, pistachios, and almonds, which are among the main production centers in the regions Crops of Astana- Ashrafieh, Taft, Estehban, Rafsanjan and Bowanat sample. After the purification of *Aspergillus*, the purified samples were transferred to CYA MEA and CY20S culture media, and after a week, they were measured and studied morphologically (Klich & Pitt, 1988). To analyze the results of macroscopic and microscopic measurements from SPSS Ver. 20 used. In the molecular study part of this research, Zhang's method was used for DNA extraction (Zhang et al., 2013), suitable primers, conditions and the content ratio of PCR compounds to conduct ISSR study according to previous studies and [researches-research](#) (Zhang et al., 2013; Al -Wadai et al., 2013; Salim et al., 2019) were optimized with a slight change. In this, PAST and GenAlex software [were](#) used to analyze the results.

Results

Based on the result of the one-way analysis of variance in SPSS software, no significant difference was observed between the macroscopic and microscopic traits of the varieties isolated from different sources ($P>0.05$). The results obtained from the ISSR study show that in the initial screening, 153 bands with 76% polymorphism were obtained

from the twenty selected primers. Primer 834 produced more bands than other primers by producing 12 bands. The analysis of the information obtained by the ISSR method in the GenAlex software shows the presence of intra-population variation equal to 31% and inter-population variation equal to 69%. The clustering analysis performed based on UPGMA in PAST software shows three distinct clusters. The population of *Aspergillus flavus* isolated from Astana-Ashrafieh peanuts is in the first cluster, the population isolated from Bowanat almonds is in the next cluster, and the third cluster includes three sub-clusters: Rafsanjan pistachio, Taft dried mulberry, and Estehban dried fig. In the PCoA analysis based on the main components, it is evident that the isolates purified from pistachio, mulberry, and fig are more closely related to the isolates purified from peanut and almond. In the CCA analysis, the environmental factors of the agricultural areas; including the average annual temperature, average annual rainfall, altitude, and latitude, are also measured and included in the calculations in addition to the results obtained from ISSR. The analysis also confirms the separation of the purified populations of pistachios, dried berries, and figs from the populations of almonds and peanuts.

Discussion

The results of this research prove the ability of the ISSR method in differentiating *A. flavus* populations obtained from different regions and different hosts. Although there have been very limited studies on the use of the ISSR method to identify *Aspergillus*

populations, the results of this research are consistent with the results of the study by Zhang et al. and Mahmoud et al. (Zhang et al., 2013; Mahmoud et al., 2016). Although the research results of Al-Wadai et al. do not strongly confirm the results of this research, but they emphasize that the ISSR method has a higher discrimination ability than the RAPD method for studying *A. flavus* populations (Al-Wadai et al., 2013). Based on the result of the CCA analysis, the most effective climatic factors in the isolation of the purified population from Bowanat almonds compared to other populations, were the average annual temperature and altitude. Average annual temperature and average annual rainfall are the most effective factors for the separation of the pure populations from the peanuts of the Astana-Ashrafieh region. Astana-Ashrafieh region has the highest average rainfall and relative humidity among the investigated regions, while the three regions of Taft, Estehban, and Rafsanjan have the lowest average rainfall among the investigated regions. Despite the fact that in terms of macroclimates such as latitude, altitude, and rainfall, there is more affinity between the Rafsanjan region of Kerman province and the Estehban region of Fars province, the analysis PCOA and UPGMA show more similarity between the populations isolated from Estehban fig and Taft mulberry. In this regard, It seems the similarity of the composition and nutrients of the host (mulberries and figs are both from the Moraceae family) is more effective than the similarity of the macroclimate, while peanuts are from the Fabaceae family and almonds are from the Rosaceae family. It is ~~suggesting-suggested earrying-to carry~~ out; more detailed research in this position; because if there is a meaningful and provable relationship, it can create a new horizon in the identification of saprophyte fungal populations.

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