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Research Article

Investigating Genetic Diversity of Foeniculum Vulgare Mill using Molecular Markers

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ABSTRACT

Medicinal plants are considered valuable genetic resources in Iran. One of these medicinal as well as spice plants is Foeniculum Vulgare Mill from Umbellifetae family used in different industries such as food, medicine, and cosmetics. It seems that due to different climate conditions in Iran this plant represents a high and valuable genetic diversity; therefore, management of genetic resources protection and obtaining information about genetic diversity will help awareness of evolution processes as well as genetic erosion of this valuable plant. Genetic diversity in local masses of Foeniculum Vulgare Mill can be investigated using molecule markers such as AFLP, RAPD, ISSR, SRAP, RFLP, and so on. In investigation of over 30 ecotype of local Foeniculum Vulgare Mill, different markers have shown that mean polymorphic content (PIC) is about 36% and mean genetic diversity is estimated about 40% in different samples. Data obtained from molecule software analyses help to categorize Foeniculum Vulgare Mill genotype in different groups based on climate and geographical conditions. Principle components analysis (PCOA) has also confirmed the results of cluster analysis. Dendrogram obtained by cluster analysis based on similarity coefficient of simple matching (SM) and UPGMA algorithm can also categorize population of Foeniculum Vulgare Mill in different groups. Results of molecular variance analysis (AMOVA) have shown that most genetic variance between geographical groups can be seen in populations. In general, according to investigations, there is a significant genetic diversity regarding agronomic and molecular traits of Foeniculum Vulgare Mill masses in Iran and knowing this genetic diversity will help in breeding programs, complementary studies, categorization, and so on.

Keywords: Foeniculum Vulgare Mill, genetic diversity, molecular markers

INTRODUCTION

Foeniculum Vulgare Mill which is from umbelliferae family is an old medicinal and spice plant in Iran used in many different industries such as food, medicine, and cosmetics (Omidbeigi, 1376; Rechinger, 1986). This plant is native to southern part of Europe and Mediterranean and it is a wild plant in France, Spain, Portugal, and North Africa. At present it is cultivated in vast areas of Romania, Russia, Germany, France, Italy, India, Argentina, America, and many African countries (Mozafarian, 1362). Moreover, Turkey, China, Syria, Iran, Vietnam, Afghanistan, Lebanon, and Cyprus are main producers of this plant (Shangmugarelu, 2008). Foeniculum Vulgare Mill is a perennial herbaceous plant with chromosome number of 28=22 (Safai, 1387). Vast germplasm resources, genetic diversity among species, low water requirement, and indifference to drought are among factors which have increased the importance of this plant (Charles, 1993).

Obtaining information on genetic interval among individuals or populations and awareness of family relationships among special species in breeding programs will provide the ability to organize germoplasm and sample genotypes effectively.The first step in improvement of plant traits is identification of genetic traits in germoplasm samples which can in turn facilitate germoplasm systematic sampling in order to improve breeding and protection (Nybom, 2008). Markers are based on PCR and include genomic DNA digestion, specific restriction enzymes, connection of short multi-nucleotide adaptors to the end of restricted pieces, and then proliferation of the resulting pieces through PCR. This technique represents difference in restriction sites. The difference in molecular markers' methods such as ISSR, SARP, AFLP, RFLP, and RAPD is that in some citrine blotting is used for representation of the pieces while in some others proliferation is done by PCR. Some markers show a combination of two or more different markers; for example, AFLP marker is a combination of RFLP and RAPD. Markers' sensitivity in polymorphic representation is different across genome. Some markers are more preferable due to higher reproducibility and also some are more preferred because they don't need primary information about the genome and their high reliability in providing polymorphic markers is an advantage over others (Farsi, 1382).

MATERIALS AND METHODS

In order to investigate genetic diversity of Foeniculum Vulgare Mill native masses, different seeds are selected from different cities and regions of Iran (with distribution of over 30 cities in Iran) in order to plant and evaluate genetic diversity. Sample selection and investigation should be performed in a way that favorable distribution is obtained from diversified cultivars. Seeds are put in hypochlorite 7% for 10 minutes and are then washed four times with distilled water (Anzidei, 2005). Plant leaves are usually used for DNA extraction. DNA extraction can be performed following Dellaporta et al with some changes and more purification. More DNA purification is improved by Chloroform Ismail Alcohol through different steps. Investigation of extracted DNA quality and quantity is done by spectrophotometry and agarose electrophoresis (Dellaporta et al, 2003). These are different stages of doing molecular markers method depending on the type of the marker; generally, first DNA genomes are restricted using special restriction enzymes especially ECORI, and then the design of mechanisms and primers is done based on sequence of the restricted position after which two types of corresponding mechanisms are connected to the end of restricted pieces. Connection of primers to restricted pieces is available through T4 DNA Ligase enzyme. Afterward, the resulting pieces undergo primary proliferation using PCR and this step can be done by different primers. Proliferated DNA pieces are separated in vertical electrophoresis device on acrylamide gels for DNA replication and coloring can be done using silver nitrate (Sanguinetti et al, 2004). In analysis of molecular markers data, each piece of proliferated DNA is considered an industry and presence and absence of bases can be evaluated manually with zero and one. Data are usually called out after entering Excel software in order to calculate similarity matrix and cluster analysis with NTSYS-PC software. According to molecular analyses in test samples, UPGMA algorithm is selected as the best method based on Jacquard similarity coefficient and due to better genetic information restoration which results in determination of genetic similarity between samples.

DISCUSSION AND CONCLUSION

Investigation of different molecular markers has shown that average polymorphic content (PIC) is about 0.36 and average genetic diversity among different samples is estimated 0.40 which can help to categorize data obtained from analyses of Foeniculum Vulgare Mill genotype molecular software based on geographical regions as well as climatic

conditions. Principal components analysis (PCOA) has also confirmed the results of cluster analysis. Dendrogram obtained by cluster analysis can categorize Foeniculum Vulgare Mill population based on simple matching coefficient (SM) and UPGMA algorithm. Results of molecular variance analysis (AMOVA) have shown that there is the most genetic variance among different geographical groups of the population. Based on cluster analysis categorization, geographical, and molecular diversity, it is obvious that according to investigated dendrogram samples collected from most cities of Iran are placed in one group and other samples are placed in groups with different genetic diversity. According to investigations, samples collected from Yazd, Gonabad, and Tabriz are each in a separate group. In this regard, most samples are placed in one group because of higher genetic similarity which is due to similar geographical conditions. Tabriz genotype shows the highest genetic diversity among others followed by Isfahan samples. Given that samples from different areas with different geographical as well as climate conditions can be obtained and investigated in Iran, the role of geographical and climate conditions can be evaluated. In most cases, genetic diversity of samples is limited in each city. The results show that molecular markers are favorable tools for disclosure of genetic diversity in different samples of Foeniculum Vulgare Mill. There is significant genetic diversity in Foeniculum Vulgare Mill masses in Iran regarding agricultural and molecular traits and awareness of this genetic diversity can help to sort and identify different genotypes of Foeniculum Vulgare Mill which eventually enables breeders to determine the highest genetic interval between parents.

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