



**University of Zabol**

Graduate school

Faculty of Agriculture

Department of Aquiculture

**The Thesis Submitted for the Degree of Master of Science**

**Plant Breeding and Biotechnology**

**Investigation the phylogenic relationships of some genotypes of  
pumkin using DNA barcoding**

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## Abstract

Many valuable medicinal species are endangered due to deforestation, expansion of agricultural activities, overgrazing and over-exploitation of livestock and over-exploitation, causing damage to the environment and reducing biodiversity. . Since any management program to improve, rehabilitate or exploit natural resources, requires knowledge of vegetation, accurate information about the quantitative and qualitative characteristics of plants and knowledge of the relationship between plants and environmental factors, so Determining the flora of medicinal plants is of particular importance in the management of natural and national resources. In order to investigate this study, 2 species and 3 genotypes of squash were studied. Pumpkin belongs to the family Cucurbitaceae, which has a variety of species, of which only 5 species are of high agricultural importance. The success of breeding programs depends on the genetic diversity of the population. Knowing the genetic distance between individuals or populations and being aware of the kinship relationships of the species in the breeding program allows the organization of hereditary storage and effective sampling of genotypes and better utilization of diversity. Markers related to the ITS region of ribosomal genes as well as markers related to the endogenous regions of DNA chloroplasts (IGS) are major markers that can be used to study genetic diversity and phylogenetic relationships. With the aim of determining the ability of IGS and ITS markers in evaluating different cultivars and genotypes of pumpkin as well as determining the genetic distance and diversity between different cultivars of pumpkin, to identify valuable cultivars or genotypes and use in breeding programs, two cultivars and three genotypes of the plant Pumpkin was evaluated. After sequencing of ITS and IGS regions and sequencing by NCBI blast software, ClustalW algorithm in MEGA6 software was used to sequence alignment. Continuing the study of genetic indicators with the help of Dnasp software, in the ITS region among all cultivars and genotypes studied, the highest percentage of Berber C + G with 59.8% and related to paper skin and with IGS marker in paper skin 47, It is 3%. Nucleotide substitution was investigated based on the Tamura-Nei model, which in both ITS and IGS regions was the most transient and purine-based substitution. The numerical value of  $dN / dS$  in the studied cultivars and genotypes was equal to 0.604 in the ITS region and 0.030 in the IGS region, which shows the net selection trend during the evolution period. 791 positions were removed and added. The highest genetic distance was determined between pairs of ITS sequence regions between paper and pen skin and in IGS between oyster and Mashhadi. By drawing a phylogenetic tree and pcoA diagram in this study, it was found that the ITS marker shows more genetic diversity and distance than the IGS marker, which can differentiate different cultivars of squash species.

**Key words:** Diversity study, Ribosomal gene, Genetic similarity, pumkin, IGS