



University of Zabol
Graduate school
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**The Thesis Submitted for Degree of Ph.D. in Biotechnology in
agriculture**

**Study of evolutionary and phylogenetic
relationships and identification candidate
miRNAs in Iranian *Allium* species**

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Abstract

Allium L. is one of the largest genera of the *Amaryllidaceae* family, with more than 920 species including many economically important species used as vegetables, spices, medicines, or ornamental plants. Currently, DNA barcoding tools are being successfully used for the molecular taxonomy of *Allium*. MicroRNAs (miRNAs) are a class of endogenous small regulatory RNAs consisting of about 18–22 nucleotides derived from their precursor sequences. In this study to investigate phylogenetic relationships using matk chloroplast marker we used a total of 46 species of *Allium*. Sequencing results and analysis of the data and generated phylogenetic tree with several method such as Neighbor-joining shown that, The NJ dendrogram generated three distinct clades: the first contained *Allium austroiranicum* and *A. ampeloprasum*; the second contained *A. iranshahrii*, *A. bisotunense*, *A. cf assadi* and *A. rubellum*. the third contained and other species. according to the rooting of the tree, the first two species are older than the other species. In this study, EST-based methods were used to identify miRNA in *Allium*. A total of 45,510 reported *Allium* EST sequences were received and after BLASTn and BLASTx with all miRbase sequences and deleting protein coding sequences, finally miR169 and miR171 sequences with 9 EST sequence of *Allium* had homology and And were selected as miRNA candidates. After designing specific primers and performing RT PCR and Real-Time PCR and band and The amplification curve observation, also indicated the presence of these miRNA sequences in *Allium*.

Keywords: *Allium*, matK, phylogeny, miRNA, EST