Transcriptome Analysis in Medicinal Plant Leaves of *Citrullus colocynthis* to Identify the Genes Related to Production of Pharmaceutical Compounds

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Abstract: *Citrullus colocynthis* L. is one of the most important medicinal plants. It is a perennial herb species of cucurbitaceae family with bitter substance called Cucurbitacin, which is the main biochemical compound of this plant. Cucurbitacins are a class of cucurbitane-type tetracyclic triterpenoids that are mainly produced by plants of the cucurbitaceae family [1], and have properties anti-diabetes, anti-cancer, anti-inflammatory and anti-fungus [2]. Triterpenoids are synthesized from mevalonic acid via the isoprenoid pathway [3]. Thefirst dedicated gene in cucurbitacin biosynthesis is cucurbitadienol synthase (CDS), which catalyses the first committed step incucurbitacin biosynthesis, i.e. the formation of the cucurbitadienol skeleton [4]. These inheritance analyses concluded that there were two loci controlling fruit bitterness in the population, one locus, bi (bi-1), making fruit and foliage bitter-free and Bt (Bt-1) making the fruit highly bitter. An additional locus was also identified; bi-3. The bi-1 locus was positioned on chromosome 6 in a region spanning 160 predicted genes. The locus of bi-3 was placed on chromosome 5 and limited to a region with nearly 200 predicted genes. Among the160 predicted genes for bi-1, an oxidosqualene cyclase (named Csa008595) was found with highest similarity to cucurbitadienol cyclase [5].In recent years, the continuing technical improvements and decreasing cost of next-generation sequencing technology have made RNA sequencing (RNA-seq) a popular choice for gene expression studies. Such sequence-based methods have revolutionized studies of the transcriptome by enabling a wide range of novel applications, including detection of alternative splicing isoforms [6,7]. This research was carried out to evaluate the transcriptome of medicinal plant leaves of *Citrullus colocynthis* by RNA sequencing on plant samples of Khuzestan region. RNA leaf extraction was done by RNAeasy plant mini kit (cot.nos.74903 and 74904) in Research Laboratory, Faculty of Agriculture, Islamic Azad University, Ahvaz Branch, and transcriptional sequencing by Beijing Genomics Institute (BGI) Company in China. The sequencing was done on an Illumina HiSeq 2500 instrument for a paired-end run of 2x150 reads. Paired-end reads assembled into contigs using Trinity software. The genes expressed in the leaf of this plant are detected with using annotation and functional classification of contigs. The results of this research are used to determine the genes expressed in the identification of the biochemical pathway of cucurbitacin and SSR marker that are important in the study of medicinal plants.

Key words: RNA Seq**;** cucurbitacin; Transcriptome**;** Triterpenoids

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