Bioinformatics Analysis of the Lycopene β-Cyclase Gene in Jujube (Ziziphus jujube Mill)

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Abstract. Lycopene β-cyclase enzyme is a key cyclase in carotenoid synthesis pathway, which catalyzes the formation of catalytic ring to synthesize α-carotene and β-carotene. In this study, the physicochemical properties, secondary structure, subcellular localization and homology of ZjLCYb were analyzed. Here, the genomic mRNA sequences of LCYb gene of jujube were downloaded from NCBI and then used to bioinformatic analysis. The ZjLCYb gene sequence was 1518 bp in length and encoded 505 amino acids. The molecular mass of ZiLCYb protein is 56.81 kD, and the isoelectric point (pI) is 7.65. Subcellular localization of ZjLCYb protein was most likely located in the chloroplast. The ZjLCYb protein has a Rossmann-fold NAD(P)H/NAD(P)(+) binding (NADB) domain and a PLN02463 specific hit. Homologous analysis showed that ZjLCYb and LCYb proteins from 17 other species contained more than 84.42% similarity, with the highest similarity being Vitis vinifera and Prunus persica. This study laid a foundation for bioinformatics analysis of carotenoid biosynthesis genes of jujube.

Keywords: Jujube, Lycopene β-cyclase gene, LCYb, Bioinformatics analysis.

1. Introduction

Jujube (Ziziphus jujube Mill.) is jujube is a plant of the genus jujube in the family riliaceae, native to the middle and lower reaches of the Yellow River in China [1]. Jujube fruit is rich in nutrition, such as protein, fat, sugar, organic acids, vitamins A, vitamins C, a variety of trace calcium and amino acids and other nutrients. In addition, jujube can fight tumors, anti-oxidation, lower blood pressure and cholesterol, improve immunity, prevent insufficient blood supply to the brain, and prevent cardiovascular disease, osteoporosis and anemia [2].

Carotenoids are compounds with nutritional properties that provide natural pigments to plants and animals. As a fat-soluble substance, humans can ingest it by consuming vegetables and fruits of various colors. They are important antioxidants and have the ability to convert into essential vitamins. As an antioxidant, carotenoids are known to reduce damage caused by free radical molecules. Ingesting large amounts of carotenoids also helps prevent cell, tissue and genetic damage. This means that they have the effect of strengthening the body's immune system against infections, reducing the risk of cancer, and protecting the heart [3-4].
Lycopene - cyclase (LCYb) catalyzes the cyclic reaction of lycopene, which is essential for the cyclization of lycopene to form β-ring cyclic end groups [5]. The genetic function of LCYb in the carotenoid pathway has been studied in several species, including tabacum, strawberry, citrus, *Hibiscus esculentus* [6] and *Cerasus humilis* [7]. At present, there are no reports on the function of LCYb gene in jujube. In this study, the sequence of jujube LCYb gene was downloaded from NCBI database, and the physicochemical properties, secondary structure, subcellular localization and homology analysis of the protein were analyzed.

2. Materials and methods

2.1. Sequence Obtain of the ZjLCYb Gene

The mRNA sequence of ZjLCYb gene were searched and obtained from The National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) for subsequent bioinformatics analysis.

2.2. Bioinformatics analysis of the ZjLCYb gene

ExPASy (http://web.expasy.org) and NCBI (https://www.ncbi.nlm.nih.gov/) were used to analyze the amino acid sequence of ZjLCYb gene, and molecular weight, isoelectric point, stability Index and hydrophobicity of ZjLCYb protein. WoLF PSORT (http://www.genscript.com/wolf-psort.html) was used to predict the subcellular localization of ZjLCYb protein. NCBI was used to predict its conserved domain. DNAStar was used to analyze the secondary structure of ZjLCYb protein. The amino acid sequence of LCYb from another 17 species were downloaded from NCBI and subjected to multiple sequence alignment using DNAMAN.

3. Results

3.1. Physical and chemical properties analysis

Sequence analysis showed that the ZjLCYb gene sequence was 1518 bp in length and encoded 505 amino acids. The molecular mass of ZjLCYb protein is 56.81 kD, and the isoelectric point (pI) is 7.65. It can be seen from figure 1 that the largest number of amino acids is leucine (Leu), accounting for 12.1%, the least is tryptophan (Trp) and cysteine (Cys), each accounting for 1.4%. The prediction formula of ZjLCYb protein was C_{2559}H_{4023}N_{695}O_{728}S_{20}, the total average hydrophilicity index was -0.142, the fat solubility index was 94.36, and the solution instability index was 37.17.

![Fig. 1 Amino acid composition of ZjLCYb.](image)

3.2. Subcellular localization, conserved domain and secondary structure analysis

The WoLF PSORT software was used to predict the subcellular location of ZjLCYb. The details of the predicted results were: chlo: 5, nucl: 3.5, cysh_nucl: 2.5, vacu: 2, cyto: 1, mito: 1, plas: 1, indicating
that ZjLCYb protein was most likely located in the chloroplast. The conserved domain of the ZjLCYb protein was analyzed by the database (CDD), and the results showed that the ZjLCYb protein contained a NADB rossmann-fold NAD (P) H/NAD (P) (+) binding (NADB) domain and PLN02463 specific (Figure 2). The results of secondary structure analysis of the protein showed that LCYb protein was hydrophilic, and the protein contained 19 alpha helices, 19 beta sheets, and 37 beta-turns (Figure 3).

![Fig. 2 The protein domain of ZjLCYb.](image)

![Fig. 3 The secondary structure of ZjLCYb.](image)

### 3.3. Homology analysis

We selected 17 species from Grapaceae, Rosaceae, Rutaceae, Papaya, Sycamore, Leguminosae and jujube for homologous comparison of LCYb proteins. The results revealed that ZjLCYb protein has high homology among fruits, vegetables, flowers and trees. It can be seen from table 1 that ZjLCYb had the highest homology with LCYb proteins of Grapaceae and Rosaceae, such as *Vitis vinifera*, *Prunus persica*, *Prunus dulcis* and *Prunus mume*, with the homology level of 89.90%. It is interesting to note that *Punica granatum* had the lowest homology with ZjLCYb protein among the 17 species, but it also reached 84.42%.

| Species                | Gene name | GenBank accession No. | Identity with LCYb (%) |
|------------------------|-----------|-----------------------|------------------------|
| *Ziziphus jujuba*      | ZjLCYb    | XP_015898141.1        | 100                    |
| *Vitis vinifera*       | VvLCYb    | XP_002275769.1        | 89.90                  |
| *Prunus persica*       | PpLCYb    | XP_007202046.1        | 89.90                  |
| *Prunus dulcis*        | PdlCYb    | VVA23946.1            | 89.90                  |
| *Prunus mume*          | PmLCYb    | XP_008240729.1        | 89.90                  |
| *Citrus sinensis*      | CsLCYb    | NP_001275849.1        | 87.13                  |
| *Carica papaya*        | CpLCYb    | ACM24791.1            | 86.34                  |
| *Theobroma cacao*      | TcLCYb    | EOY09059.1            | 86.17                  |
| *Glycine max*          | GmLCYb    | XP_003554132.         | 86.14                  |
| *Herrania umbratica*   | HuLCYb    | XP_021282724.1        | 85.97                  |
| *Cephalotus follicularis* | CjLCYb   | GAV81497.1            | 85.38                  |
| *Vigna unguiculata*    | VuLCYb    | XP_027936931.1        | 84.95                  |
| *Ipomoea batatas*      | IbLCYb    | BAW34173.1            | 84.75                  |
| *Abrus precatorius*    | ApLCYb    | XP_027349790.1        | 84.75                  |
| *Corchorus olitorius*  | CoLCYb    | OMO60094.1            | 84.65                  |
| *Corchorus capsularis* | CcLCYb    | OMO58144.1            | 84.65                  |
| *Syzygium oleosum*     | SoLCYb    | XP_030453081.1        | 84.55                  |
| *Punica granatum*      | PgLCYb    | XP_031387768.1        | 84.42                  |
4. Discussions
The bioinformatics analysis of ZjLCYb gene in jujube tree was carried out by online software. In both Hibiscus esculentus [6] and jujube, the LCYb enzyme is encoded by a single copy of the gene, suggesting that the enzyme may have undergone a similar evolutionary pattern and that it plays an essential role in the carotenoid metabolism of the jujube. Previous studies have reported that LCYb protein is relatively conservative in plants [6]. The LCYb protein of Hibiscus esculentus is similar to the LCYb protein of Gossypium hirsutum, with a homology of 91% [6]. The results of this study showed that jujube LCYb was highly conserved, especially in the Rosaceae, with homology of about 90%. This study laid a foundation for bioinformatics analysis of carotenoid biosynthesis genes of jujube.

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