Bioinformatics Analysis of the 1-Deoxy-D-Xylulose-5-Phosphate Synthase (DXS) Gene in Cabbage (Brassica Oleracea Var. Capitata)

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Abstract. 1-deoxy-D-xylulose-5-phosphate synthase (DXS) is an rate-determing enzyme in carotenoid biosynthesis. Here, the Brassica oleracea var. capitata DXS (BocDXS) gene sequences were obtained from Brassica database (BRAD), and preformed for bioinformatics analysis. The BocDXS1, BocDXS2 and BocDXS3 genes mapped to chromosomes 3, 7 and 9, and contains an open reading frame of 1,893 bp, 2,049 bp and 2,088 bp that encodes a 712, 682, 695 amino acid protein, respectively. Subcellular localization predicted all BocDXS genes were in the chloroplast. The conserved domain of the BocDXS protein is PLN02582. Homology analysis indicates that the DXS protein is apparently conserved during plant evolution. The findings of the present study provide a molecular basis for the elucidation of DXS gene function in cabbage.

1. Introduction

Cabbage (Brassica oleracea var. capitata) is a member of the Brassicaceae family that is widely distributed in the world. In China, cabbage is an important vegetable crop, and consumed considerable every year. Cabbage is generally grown for its leafy head as common edible part, which are crispy, tender, and tasty [1]. Besides its good flavor, cabbage is also a rich source of nutrients, antioxidants, and anticarcinogenic compounds, including carbohydrates, vitamin C, carotenoids, and glucosinolates [1-2].

Carotenoids, which are synthesized in various photosynthetic and non-photosynthetic organisms, including algae, plants, and some bacteria and fungi, are a class of 40-carbon hydrocarbon compounds derived from a terpenoid precursor [3]. The enzymes involved in the carotenoid biosynthetic pathway have been extensively studied in various plants, including Arabidopsis [4], tomato [5], and citrus [6]. The first key step in carotenoid biosynthesis involves the production of a 40-carbon phytoene from two geranylgeranyl pyrophosphate (GGPP) molecules, which is catalyzed by phytoene synthase (PSY) [7-8]. Then, lycopene (colored carotenoid) is converted from phytoene (non-color carotenoid) by desaturases and isomerases, including phytoene desaturases (PDS) [9], ζ-carotene desaturase (ZDS) [10], 15-cis-ζ-carotene isomerase (Z-ISO) [11], and carotenoid isomerase (CRTISO) [4]. Hereafter, bifurcation of the carotenoid biosynthetic pathway occurs, and the production of β-carotene and α-carotene is catalyzed by lycopene β-cyclase (LCYb) and lycopene ε-cyclase (LCYe) [12-13].

Carotenoid biosynthesis in plants is derived from the methylerythritol 4-phosphate (MEP) pathway. The MEP pathway uses glyceraldehyde 2-phosphate and pyruvate as initial substrates to form1-deoxy-D-xylulose-5-phosphate (DXP), which is catalyzed by DXP synthase (DXS) [3].The genes encoding the DXS protein have been isolated in various plant species, including Arabidopsis, tomato, maize and potato [3,14]. To date, research studies on DXS in cabbage are limited. In the present study, the DXS gene sequence of cabbage was obtained from web database, and then bioinformatics analysis of the DXS gene were analyzed. The present study aimed to establish the foundation for further studies on the molecular mechanism of DXS in cabbage.

2. Materials and Methods

2.1 Sequence Obtain of the Bocdxs Gene.

The genomic DNA and mRNA sequences of DXS gene of cabbage were downloaded and obtained from The Brassica database (BRAD) (http://brassicadb.org), and then used to subsequent bioinformatic analysis.

2.2 Bioinformatics Analysis of the BocDXS Gene.

The amino acid sequence, protein molecular weight, isoelectric point, stability index, and hydrophobicity of the BocDXS gene were analyzed and predicted by ExPASy (http://web.expasy.org) and NCBI (https://www.ncbi.nlm.nih.gov/). Subcellular localization was predicted by WoLF PSORT (http://www.genscript.com/wolf-psort.html). The conserved domain were predicted by NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Phylogenetic tree analysis of the DXS proteins was executed in MEGA 6.0 using the neighbor-joining (NJ) method.

3. Results

3.1 Analysis on Genomic Organization.

The Brassica database (BRAD) was used to analyze the chromosomal localization and genomic organization of BocDXS. There are three genes of DXS in cabbage chromosomes, BocDXS1, BocDXS2 and BocDXS3, and the gene IDs in BRAD are Bol026609, Bol005061 and Bol043580, respectively. The BocDXS1 gene was mapped to chromosomes 3 and has 9 exons and 8 introns, the BocDXS2 gene was mapped to chromosomes 7 and has 8 exons and 7 introns, and the BocDXS3 gene was mapped to chromosomes 9 and has 10 exons and 9 introns (Fig. 1).

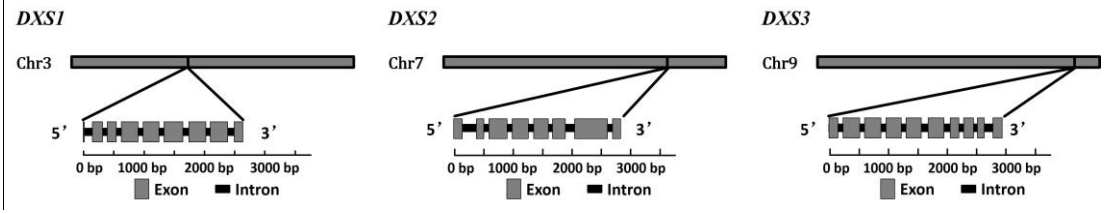

Fig. 1. Chromosomal location and genomic structure of BocDXSs.

3.2 Protein Physical and Chemical Properties Analysis.

Sequence analysis indicated that the BocDXS1, BocDXS2 and BocDXS3 gene contained a 1,893 bp, 2,049 bp and 2,088 bp open reading frame (ORF), which encoded a 712, 682, 695 amino acids protein with a calculated molecular mass of 77.21 kD, 72.99 kD and 75.84 kD, and an isoelectric point (pI) of 8.59, 6.86 and 6.99, respectively. The amino acid types and proportions of the BocDXSs gene was shown in Figure 2, the highest number of amino acid in BocDXS1 and BocDXS3 is Leucine (Leu), the highest number in BocDXS2 is Alanine (Ala), whereas the lowest number in each gene is Tryptophan (Trp). The predicted formula of BocDXS1, BocDXS2 and BocDXS3 were C3417H5418N962O1019S29, C3216H5117N905O977S28 and C3352H5376N938O1002S31, respectively. Their total average hydrophilicity index were -0.16, -0.139 and -0.013, liposoluble index were 85.07, 86.14 and 95.41, and instability index in solution were 36.69, 38.82 and 38.13, respectively. None of the three genes have a transmembrane structure (Fig. 3).

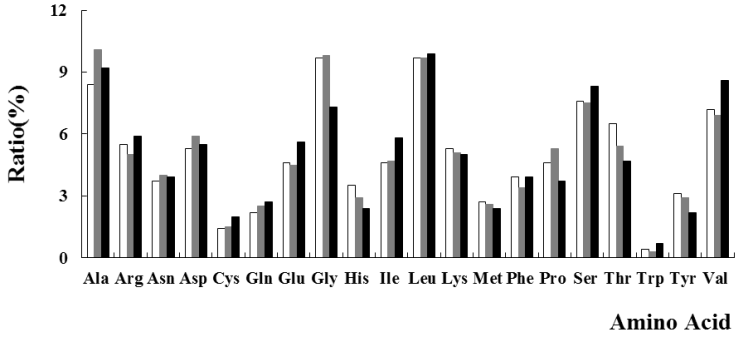

Fig. 2. Amino acid composition of BocDXSs

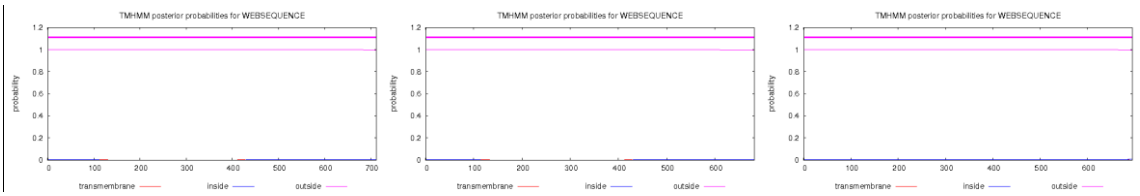

Fig. 3. Prediction of protein transmembrane structure of BocDXSs

3.3 Subcellular Localization and Conserved Domain Analysis.

Subcellular localization of the BocDXS1, BocDXS2 and BocDXS3 were all predicted by WoLF PSORT to be in the chloroplast. The analysis using Conserved Domain Database (CDD) demonstrated that the amino acid sequence of all BocDXS proteins have the conserved domain PLN02582 and Transketolase_C superfamily.

3.4 Homology and Phylogenetic Tree Analysis.

A phylogenetic tree was constructed to illustrate the relationship among the DXS proteins of cabbage and 21 other higher plant species (Fig. 4). A total of three major clusters were identified, BocDXS2 belongs the first cluster, and BocDXS1 and BocDXS3 belong the second cluster. Sequence alignment indicated that the BocDXS1 protein is more closely related to B. napus, BocDXS2 protein is more closely related to B. rapa and Solanum lycopersicum, and BocDXS3 protein is more closely related to Eutrema salsugineum.

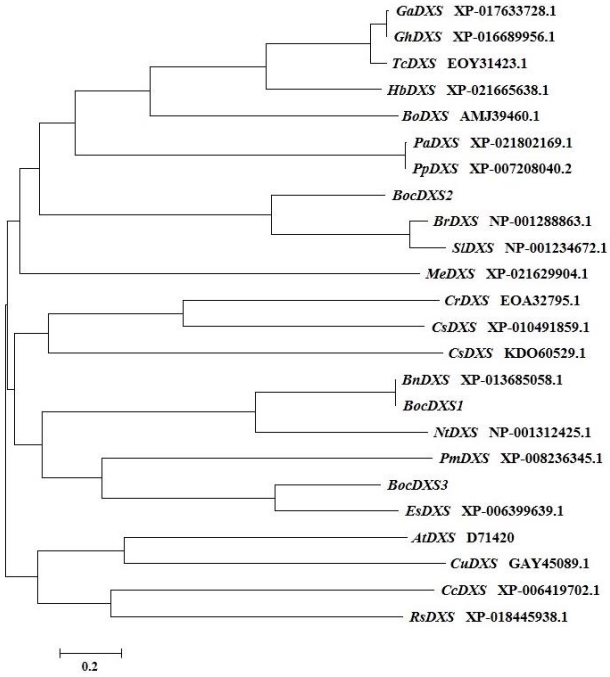

Fig. 4. Phylogenetic tree analysis of BocDXSs and DXS proteins of other species

4. Summary

The present study analyzed the BocDXS genes of cabbage. DXS is important in carotenoid flux regulation. The enzyme is encoded by a single gene and appear to be rate-determing enzyme in Arabidopsis thaliana. Overexpression of DXS in Arabidopsis seedlings increases carotenoid production [3]. The findings of the present study show that DXSs from cabbage are highly conserved, particularly in the Cruciferae, similar to that observed in earlier reports. The findings of the present study may serve as a foundation for future studies on the functions of DXSs in carotenoid metabolism in cabbage.

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