Bioinformatics Analysis of the Z-Carotene Isomerase Gene in Cabbage (Brassica Oleracea Var. Capitata)

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Abstract. Z-carotene isomerase (Z-ISO) is an important enzyme in carotenoid biosynthesis. Here, the *Brassica oleracea* var. *capitata Z-ISO* (*BocZ-ISO*) gene sequence was obtained from *Brassica* database (BRAD), and preformed for bioinformatics analysis. The *BocZ-ISO* gene mapped to chromosomes 5, and contains an open reading frame of 1,092 bp that encodes a 363-amino acid protein with a calculated molecular mass of 40.24 kD and an isoelectric point (pI) of 9.56. Subcellular localization predicted the *BocZ-ISO* gene was in the chloroplast. The conserved domain of the BocZ-ISO protein is COG4094. The Z-ISO protein is most closely related to *B. napus* and *B. rapa*. The findings of the present study provide a molecular basis for the elucidation of *Z-ISO* 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 years. 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, glucosinolates, and carotenoids [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 (β-LCY) and lycopene ε-cyclase (ε-LCY) [12-13].

Z-ISO is essential for the cis-to-trans conversion of the 15-cis-bond in 9,15,9'-tri-cis-ζ-carotene (the product of PDS) to 9,9'-di-cis-ζ-carotene, the substrate of ZDS [3].

The genes encoding the Z-ISO protein have been isolated in various plant species, including Arabidopsis, maize and Osmanthus fragrans [14]. To date, research studies on Z-ISO in cabbage are limited. In the present study, the Z-ISO gene sequence of cabbage was obtained from web database, and then bioinformatics analysis of the Z-ISO gene were analyzed. The present study aimed to establish the foundation for further studies on the molecular mechanism of Z-ISO in cabbage.

2. Materials and Methods

2.1 Sequence Obtention of the BocZ-ISO Gene

The genomic DNA and mRNA sequences of Z-ISO 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 BocZ-ISO Gene

The amino acid sequence, protein molecular weight, isoelectric point, stability index, and hydrophobicity of the BocZ-ISO 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 Z-ISO 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 BocZ-ISO. The gene ID in BRAD is Bol036691. The BocZ-ISO gene was mapped to chromosomes 5 and has 4 exons and 3 introns (Fig. 1).

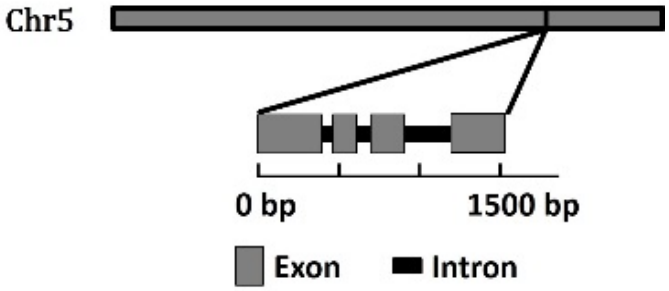

Fig. 1 Chromosomal location and genomic structure of BocZ-ISO.

3.2 Protein Physical and Chemical Properties Analysis

Sequence analysis indicated that the BocZ-ISO gene contained a 1,092-bp open reading frame (ORF), which encoded a 363-amino acids protein with a calculated molecular mass of 40.24 kD and an isoelectric point (pI) of 9.56. The amino acid types and proportions of the BocZ-ISO gene was shown in Figure 2, the highest number of amino acid is Leucine (Leu), whereas the lowest number is Cysteine (Cys). Its predicted formula was C1863H2891N487O490S9. Its total average hydrophilicity index was 0.288, liposoluble index was 106.12, and instability index in solution was 43.15.

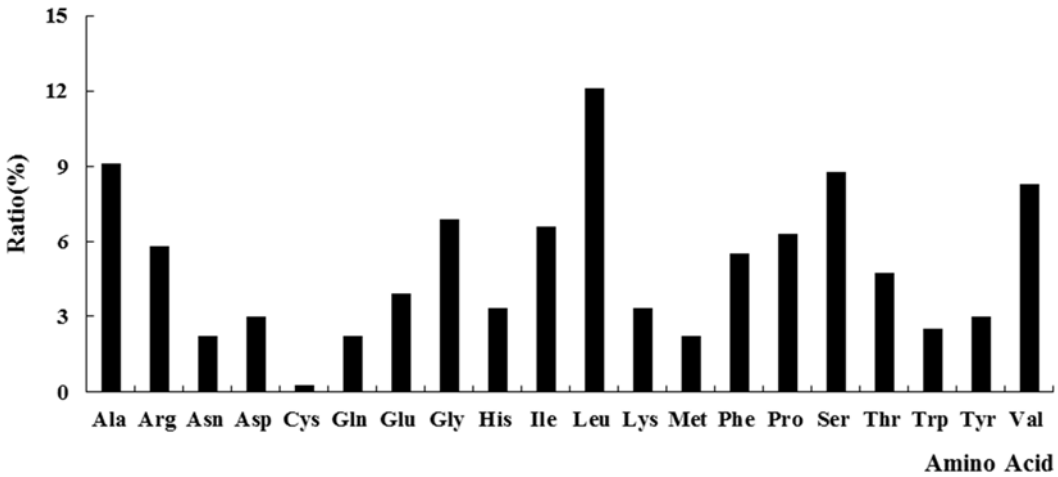

Fig. 2 Amino acid composition of BocZ-ISO

3.3 Subcellular Localization and Conserved Domain Analysis

Subcellular localization of the BocZ-ISO gene was predicted by WoLF PSORT to be in the chloroplast. The analysis using Conserved Domain Database (CDD) demonstrated that the amino acid sequence of the BocZ-ISO protein has one conserved domain COG4094 and one NnrU superfamily.

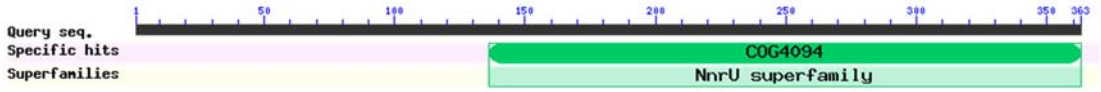

Fig. 3 Conserved domains analysis of BocZ-ISO

3.4 Homology and Phylogenetic Tree Analysis

A phylogenetic tree was constructed to illustrate the relationship among the Z-ISO proteins of cabbage and 19 other higher plant species (Fig. 4). A total of two major clusters were identified, one cluster includes Cruciferae and Rosaceae, while the other cluster includes Leguminosae, Solanaceae, Sterculiaceae, Cruciferae, and Malvaceae. Sequence alignment indicated that the BocZ-ISO protein is more closely related to B. napus and B. rapa, which belonged to the Brassica branch.

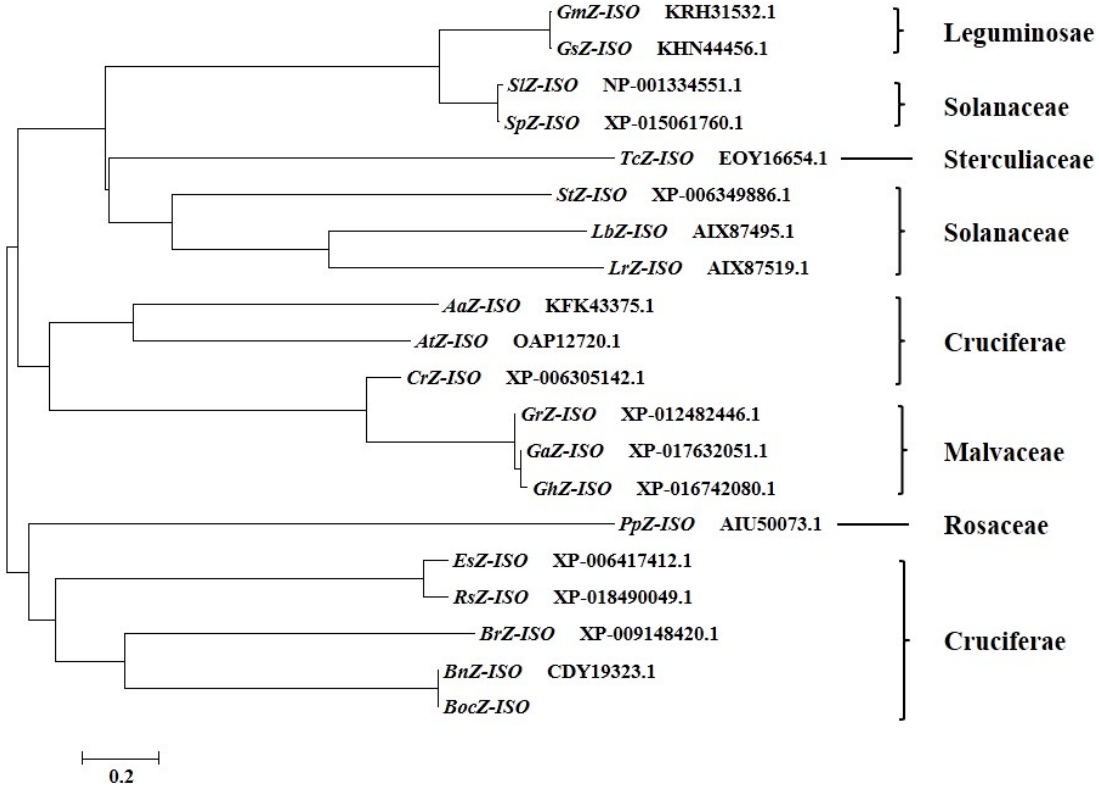

Fig. 4 Phylogenetic tree analysis of BocZ-ISO and Z-ISO proteins of other species

4. Discussion

The present study analyzed the *BocZ-ISO* gene of cabbage. Z-ISO enzyme is encoded by two-copy genes in *Osmanthus fragrans* [14]. However, the *Z-ISO* gene occurred as a single copy in cabbage, indicating that the enzyme may have undergone different evolutionary patterns. Previous studies have shown that the Z-ISO protein is relatively conserved in plants [14]. The Z-ISO1 protein of *Osmanthus fragrans* is similar to the Z-ISO protein of *Zea mays* and *Arabidopsis thaliana*, showing 61.56% and 64.08% homology [14]. The findings of the present study show that Z-ISO from cabbage is 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 Z-ISO in carotenoid metabolism in cabbage.

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