

Bioinformatics Analysis of the Carotenoid Isomerase Gene in Cabbage (*Brassica oleracea* var. *capitata*)

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Abstract. Carotenoid isomerase (CRTISO) is an important enzyme in carotenoid biosynthesis. Here, the *Brassica oleracea* var. *capitata* CRTISO (BocCRTISO) gene sequences were obtained from Brassica database (BRAD), and preformed for bioinformatics analysis. The BocCRTISO gene mapped to Scaffold000330, and contains an open reading frame of 1,773 bp that encodes a 590-amino acid protein with a calculated molecular mass of 64.99 kD and an isoelectric point (pI) of 6.75. Subcellular localization predicted the BocCRTISO gene was in the chloroplast. The conserved domain of the BocCRTISO protein is Rossmann-fold NAD (P)H/NAD(P)(+) binding (NADB) domain. Homology analysis indicates that the CRTISO protein is apparently conserved during plant evolution and is most closely related to *B. napus*, and *B. rapa*. The findings of the present study provide a molecular basis for the elucidation of CRTISO gene function in cabbage.

Keywords: Carotenoid isomerase; gene sequences; bioinformatics.

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].

The enzymes involved in the carotenoid biosynthetic pathway have been extensively studied in various plants, including *Arabidopsis* [3], tomato [4], and citrus [5]. 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) [6-7]. Then, lycopene (colored carotenoid) is converted from phytoene (non-color carotenoid) by desaturases and isomerases, including phytoene desaturases (PDS) [8], ζ -carotene desaturase (ZDS) [9], 15-cis- ζ -carotene isomerase (Z-ISO) [10], and carotenoid isomerase (CRTISO) [3]. 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) [11-12].

CRTISO is an important enzyme in carotenoid biosynthesis, catalyzing the prolycopene into lycopene [13]. It is reported the identification of a CRTISO as the candidate gene for orange head by high-resolution genetic mapping using F2S4 population [14]. Loss of BrCRTISO function, upregulation of the upstream genes, and downregulation of downstream genes lead to the accumulation of prolycopene and confer an orange color to the inner head leaves in Chinese cabbage [15]. The genes encoding the CRTISO protein have been isolated in various plant species, including *Arabidopsis* [3], tomato [4], *N. tabacum* [16], and *B. rapa* [14]. To date, research studies on CRTISO in cabbage are limited. In the present study, the CRTISO gene sequence of cabbage was obtained from web database, and then bioinformatics analysis of the CRTISO gene were analyzed. The present study aimed to establish the foundation for further studies on the molecular mechanism of CRTISO in cabbage.

2. Materials and Methods

2.1 Sequence Obtain of the BocCRTISO Gene

The genomic DNA and mRNA sequences of CRTISO 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 BocCRTISO Gene

The amino acid sequence, protein molecular weight, isoelectric point, stability index, and hydrophobicity of the BocCRTISO 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>). The amino acid sequence of CRTISO from eight additional species were downloaded from NCBI and subjected to multiple sequence alignment using DNAMAN. Phylogenetic tree analysis of the CRTISO proteins was executed in MEGA 6.0 using the neighbor-joining (NJ) method.

3. Results

3.1 Analysis on Genomic Organization

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

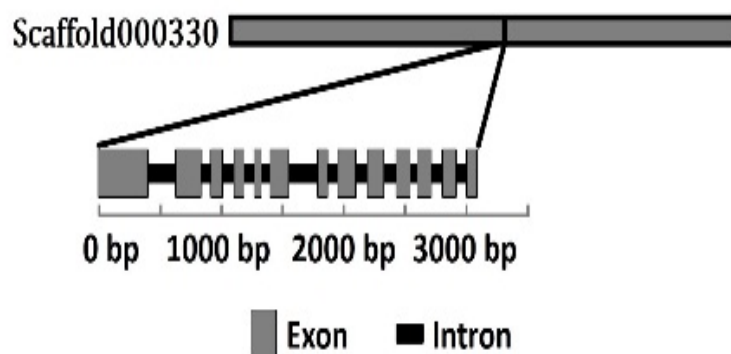


Figure 1. Chromosomal Location and Genomic Structure of BocCRTISO.

3.2 Protein Physical and Chemical Properties Analysis

Sequence analysis indicated that the BocCRTISO gene contained a 1,773-bp open reading frame (ORF), which encoded a 590-amino acids protein with a calculated molecular mass of 64.99 kD and an isoelectric point (pI) of 6.75. The amino acid types and proportions of the BocCRTISO gene was shown in Figure 2, the highest number of amino acid is Leucine (Leu), whereas the lowest number is Tryptophan (Trp). Its predicted formula was C₂₉₄₄H₄₆₁₂N₇₆₈O₈₅₁S₁₉. Its total average hydrophilicity index was -0.079, liposoluble index was 92.85, and instability index in solution was 31.35.

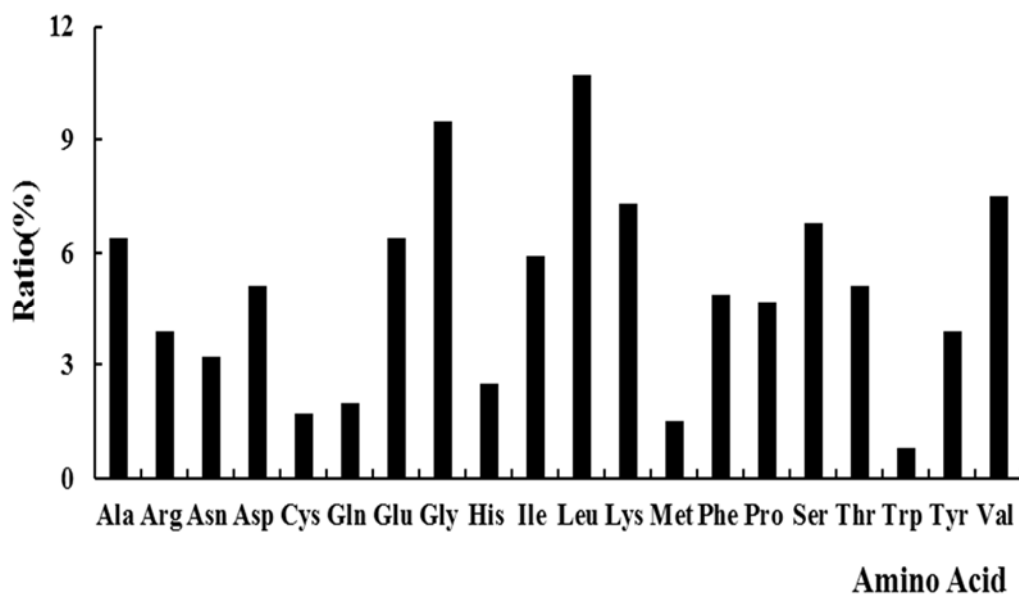


Figure 2. Amino acid Composition of BocCRTISO.

3.3 Subcellular Localization and Conserved Domain Analysis

Subcellular localization of the BocCRTISO 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 BocCRTISO protein has one NADB Rossmann superfamily that share a Rossmann-fold NAD(P)H/NAD(P)(+) binding (NADB) domain.

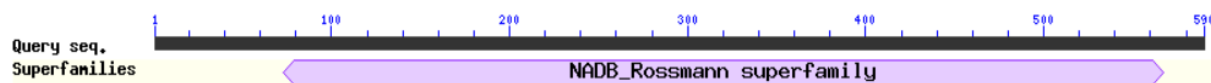


Figure 3. Conserved Domains Analysis of BocCRTISO.

3.4 Homology and Phylogenetic Tree Analysis

Homology analysis demonstrated that the amino acid sequence of the BocCRTISO protein shared high homology with those of other higher plant species. Figure 4 shows that the BocCRTISO had the highest identities with several CRTISO proteins of Cruciferae such as *B. napus*, *B. rapa*, *Arabidopsis thaliana*, and the levels of identity were 98%, 97%, 88% respectively. Interestingly, BocCRTISO showed >76% identity with other species cited in our study, indicating that the CRTISO protein is highly conserved among different species. In addition, significant differences were found near the N-termini of CRTISO proteins of various plant species (Fig. 4).

A phylogenetic tree was constructed to illustrate the relationship among the CRTISO proteins of cabbage and 19 other higher plant species (Fig. 5). A total of two major clusters were identified, one cluster includes Cruciferae, Euphorbiaceae, Sterculiaceae, and Moraceae, while the other cluster includes Solanaceae, Pedaliaceae, Rutaceae, and Rosaceae. Sequence alignment indicated that the BocCRTISO protein is more closely related to *B. napus* and *B. rapa*, which belonged to the Brassica branch.

BocCRTISO	MNLCIRNF.VTCADRSSSLFSALKTSNY...KLGTS.....KFGFLK.....HKKKNHVTVRVSS...TAVVEERTKRENG	66
BrCRTISO	MNLCIRNF.VTCADRSSSLFSALKTSNN...KLGTS.....KFGFLK.....NRKKNHVAVRSVS...STAVEERTKRESG	65
BnCRTISO	MNLCIRNF.VTCADRSSSLFSALKTSNN...KLGTS.....KFGFLK.....NPKKNHVAVRSVS...TAVVEERTKRESG	66
AtCRTISO	MDLCFQNP.VKCGDR...LFSALNTSTY...YKLGTS.....NLGFNGP...VLENRKKKKLPRMVTIKSVSSSVVASTVQGTKR	71
CtCRTISO	MNLCQSP.VECGHRSS.SFSALKTSNFGCYKLGSS.....NLGFHVR...VFENRKTQPKVTIVNSVSSAVGTVERTERDERR	75
EsCRTISO	MNLCQSP.VKCGDRSS.LFSALKTSNFGSEERLTISR.....FWGTHG.....NRKKNHVAVRSVS...SSAVGTVETETREDEIS	72
MnCRTISO	MAELCFPLC.FSPFHNSAHLITFKHSHGVQLKRIIPRSQKRRISLSITPESKLLHVGLPSSNPRQKNCKFIVRSSSVVNLDKAVERG	89
SiCRTISO	MDIMSSGLSPFQSTISDNKIRLGNCKSRNFWQKVVYCFDQWKP.NEGDHQIHGLEFGVKRAKRRQRNRFPLKSVVVDGKVLTESSSTE	89
JcCRTISO	MSLGSNTT.LQLSGFRPGIAETRLSRNFSRISFN.....CKKHG.....KVKSESFQLNKDLFSLRALSVDKEDVDIEGING	72
Consensus	m g	
BocCRTISO	G.GESKVDYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	155
BrCRTISO	G.GESKVDYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	154
BnCRTISO	G.GESKVDYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	155
CtCRTISO	D.GGESVYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	160
EsCRTISO	..GGESVYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	163
MnCRTISO	G.GESRVYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	161
SiCRTISO	G.GECKYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	178
JcCRTISO	GDRNSFYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	179
Consensus	GVTKEHRYDAIVIGSGIGGLVAATQIAVKEARVIMLEKYLIPGGSSGMYERIGTTFDVGSSVMFGFSDKGNLNLITQALAVGGRMDEVIP	162
Consensus	yda i gsg gglvaatql vk a vl leky ipggssg y r g tfdvgssvmfgfsdkgnlnlitolqal avg m vip	
BocCRTISO	DPTTVHFELENDLSVQVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	245
BrCRTISO	DPTTVHFELENDLSVQVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	244
BnCRTISO	DPTTVHFELENDLSVQVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	245
AtCRTISO	DPTTVHFELENNLSVRVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	250
CtCRTISO	DPTTVHFELENDLSVQVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	253
EsCRTISO	DPTTVHFELENNLSVRVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	251
MnCRTISO	DPTTVHFELENNLSVRVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	268
SiCRTISO	DPTTVHFELENDLSVQVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	269
JcCRTISO	DPTTVHFELENNLSVRVREYDDFVNEILSKFPHEKEGILFYGYCCKWIFNSLNSLELKSLEEHYILFGQFFCKPLECILLAYILPQNAG	252
Consensus	dpttvhf lp h ey f el kfphe gil fy cwkif lnslelksleep yilfgqff kpleciltlayilpqnag	
BocCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	335
BrCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	334
BnCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	335
CtCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	340
EsCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	343
MnCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	341
SiCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	358
JcCRTISO	DIARYIKDFCLLSFIDAECFIVSTVNAIQTPINASMVLCDREYGGINYPVGGVGIARSLAGGLVIQGSIIHYANVKSIIILIDGKAV	359
Consensus	lar yikdp llsfidaecfivstvna qtp ina mv cdrh gginyppvgv g ia sla gl qgs i y an ii g av	342
BocCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	425
BrCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	424
BnCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	425
AtCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	430
CtCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	433
EsCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	431
MnCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	448
SiCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	449
JcCRTISO	GVSLDGEFEFAKTIISNATRWDTFGKLLGEERLEEDENFQKYVYKAPSFSLSIHGVKAVLVBPTDCHHFVLEDDWKNLEBPYGSIFL	432
Consensus	gv l d r aktiisnatrwdtfgkllk lp ee fqk yvkapsfslsih gvka vl p tdchhfvl e w le pygsifl	
BocCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	515
BrCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	514
BnCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	515
AtCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	520
CtCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	523
EsCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	521
MnCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	538
SiCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	539
JcCRTISO	SIPTLLDLSLAPGGRHILHIFTTSSIEDWEGLTFKQYDEKKEDVAAGITCRLEKKLFPGLSSTITFEVGTERTHRRYLARDKGTGYGMP	522
Consensus	sipt ld lap g hilhiffts ie wegl tfkqy de kke va i rl kklfpgl ssi f evg p thr lard gtygmp	
BocCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	590
BrCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	589
BnCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	590
AtCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	595
CtCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	598
EsCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	596
MnCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	613
SiCRTISO	RGTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	614
JcCRTISO	RNTPKGLLCMFNTTIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRAVAADGLBRKSKVLDAGLLGLGLWRLTLA.....	597
Consensus	r p kgl l m f n t t i d g l y c v g d s c f p g q g v i a v a f s g v m c a h r a v a a d g l b r k s k v l d a g l l g l w r l t l a	

Figure 4. Amino Sequence Alignment of BocCRTISO with the CRTISO Protein of other Species.

Boc: Brassica oleracea var. capitata; Br: Brassica rapa(XP_009118478.1); Bn: Brassica napus(CDY09954.1); At: Arabidopsis thaliana(NP_172167.2); Cr: Capsella rubella(XP_006307051.1); Es: Eutrema salsugineum(XP_006417877.1); Mn: Morus notabilis(XP_010110449.1); Si: Sesamum indicum(XP_011077785.1); Jc: Jatropha curcas(XP_012074260.1).

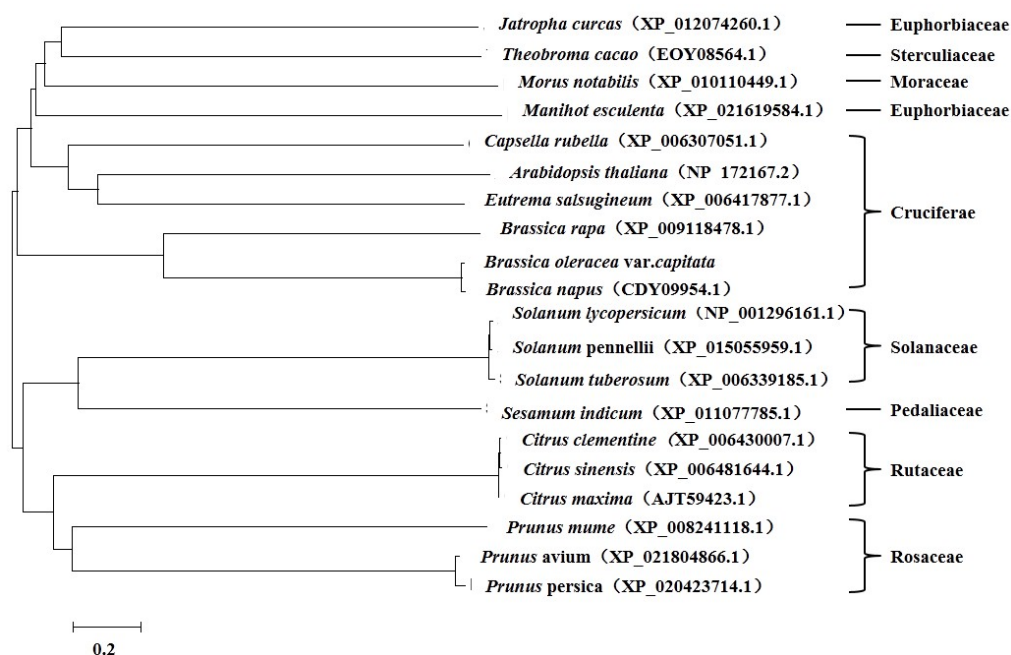


Figure 5. Phylogenetic Tree Analysis of BocCRTISO and CRTISO Proteins of other Species

4. Discussion

The present study analyzed the BocCRTISO gene of cabbage. CRTISO enzyme is encoded by single-copy genes in *Arabidopsis* [3], tomato [4], Chinese cabbage [14], and *N. tabacum* [16]. Similarly, the CRTISO gene occurred as a single copy in cabbage, indicating that the CRTISO enzyme may have undergone similar evolutionary patterns [17]. Previous studies have shown that the CRTISO protein is relatively conserved in plants [16]. The CRTISO protein of *N. tabacum* is similar to the CRTISO protein of tomato and potato, showing 93% and 93% homology [16]. The findings of the present study show that CRTISO from cabbage is highly conserved, particularly in plants; in the Cruciferae, these proteins show >88% homology, 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 CRTISO in carotenoid metabolism in cabbage.

Acknowledgments

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References

- [1]. M. Wennberg, J. Ekvall, K. Olsson, and M. Nyman, Changes in carbohydrate and glucosinolate composition in white cabbage (*Brassica oleracea* var. *capitata*) during blanching and treatment with acetic acid, *Food Chem.* 95 (2006) 226-236.
- [2]. S. Rokayya, C.J. Li, Y. Zhao, Y. Li, and C.H. Sun, Cabbage (*Brassica oleracea* L. var. *capitata*) phytochemicals with antioxidant and anti-inflammatory potential, *Asian Pac. J. Cancer Prev.* 14 (2014) 6657-6662.
- [3]. H. Park, S.S. Kreunen, A.J. Cuttriss, D. Della Penna, and B.J. Pogson, Identification of carotenoid isomerase provides insight into carotenoid biosynthesis, prolamellar body formation, and photomorphogenesis, *Plant Cell* 14 (2002) 321-332.

- [4]. T. Isaacson, G. Ronen, D. Zamir, and J. Hirschberg, Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of b-carotene and xanthophylls in plants, *Plant Cell* 14 (2002) 333-342.
- [5]. M. Kato, Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo, and M. Yano, Accumulation of carotenoids and expression of carotenoid biosynthetic genes during maturation in citrus fruit, *Plant Physiol.* 134 (2004) 824-837.
- [6]. P.A. Scolnik, and G.E. Bartley, Nucleotide sequence of an Arabidopsis cDNA for phytoene synthase, *Plant Physiol.* 104 (1994) 1471-1472.
- [7]. P.R. Li, S.J. Zhang, S.F. Zhang, F. Li, H. Zhang, F. Cheng, J. Wu, X.W. Wang, and R.F. Sun, Carotenoid biosynthetic genes in Brassica rapa: comparative genomic analysis, phylogenetic analysis, and expression profiling, *BMC Genomics* 16 (2015) 492.
- [8]. P.A. Scolnik, and G.E. Bartley, Phytoene desaturase from Arabidopsis, *Plant Physiol.* 103 (1993) 1475.
- [9]. P.A. Scolnik, and G.E. Bartley, Nucleotide sequence of zeta-carotene desaturase (accession no. U38550) from Arabidopsis, *Plant Physiol.* 109 (1995) 1499.
- [10]. Y. Chen, F. Li, and E.T. Wurtzel, Isolation and characterization of the Z-ISO gene encoding a missing component of carotenoid biosynthesis in plants, *Plant Physiol.* 153 (2010) 66-79.
- [11]. B. Pogson, K.A. McDonald, M. Truong, G. Britton, and D. Della Penna, Arabidopsis carotenoid mutants demonstrate that lutein is not essential for photosynthesis in higher plants, *Plant Cell* 8 (1996) 1627-1639.
- [12]. Y.M. Shi, R. Wang, Z.P. Luo, L.F. Jin, P.P. Liu, Q.S. Chen, Z.F. Li, F. Li, C. Y. Wei, M.Z. Wu, P. Wei, H. Xie, L.B. Qu, F.C. Lin, and J. Yang, Molecular cloning and functional characterization of the lycopene ϵ -cyclases gene via virus-induced gene silencing and its expression pattern in *Nicotiana tabacum*, *Int. J. Mol. Sci.* 15 (2014) 14766-14785.
- [13]. J.X. Zhang, H. Yuan, Z.J. Fei, B.J. Pogson, L.G. Zhang, L. Li, Molecular characterization and transcriptome analysis of orange head Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*), *Planta* 241 (2015) 1381-1394.
- [14]. J.X. Zhang, H.X. Li, M.K. Zhang, M Hui, Q. Wang, L. Li, L.G. Zhang, Fine mapping and identification of candidate BR-or gene controlling orange head of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*), *Mol Breed* 32 (2013) 799-805.
- [15]. T.B. Su, S.C. Yu, J. Wang, F.L. Zhang, Y.J. Yu, D.S. Zhang, X.Y. Zhao, W.H. Wang, Loss of function of the carotenoid isomerase gene BrCRTISO confers orange color to the inner leaves of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*), *Plant Mol. Biol. Rep.* 33 (2015) 648-659.
- [16]. Y.M. Shi, R. Wang, J. Yang, Z.P. Luo, F. Li, M.Z. Wu, C.Y. Wei, F.C. Lin, L.B. Qu, P. Wei, Cloning and functional analysis of the CRTISO gene in *Nicotiana tabacum*, *Acta Tabacaria Sinica* 20 (2014) 138-143.
- [17]. B. Sun, F. Zhang, X. Xia, S.L. Xue, Q. Yuan, Q. Chen, H.R. Tang, Cloning and expression analysis of BaPDS1 and BaPDS2 in *Brassica alboglabra*, *Acta Horticulturae Sinica* 43 (2016) 2257-2265.