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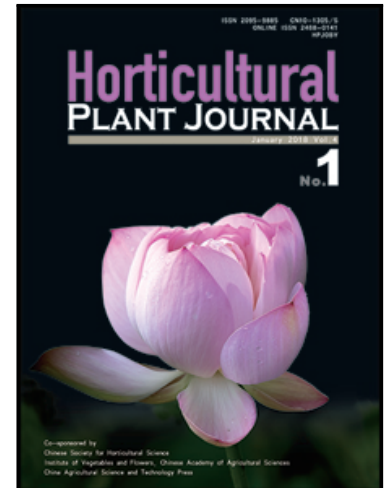
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Molecular Cloning and Expression Analysis of the ζ -Carotene Desaturase Gene in Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey)

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Abstract

ζ -carotene desaturase (ZDS) is an important enzyme in carotenoid biosynthesis. Here, the *Brassica oleracea* var. *alboglabra* ZDS (*BoaZDS*) gene was cloned from Chinese kale via reverse transcription-polymerase chain reaction (RT-PCR) and deposited in GenBank (Accession Number KY662297). The *BoaZDS* gene contains an open reading frame of 1 686 bp that encodes a 561-amino acid protein. Sequence analysis indicates that the ZDS protein is apparently conserved during plant evolution and is most closely related to *B. oleracea* var. *capitata* and *B. rapa*. The promoter sequence of the *BoaZDS* gene was predicted to harbor several *cis*-acting elements that are related to light and phytohormone responses. Semi-quantitative RT-PCR analysis showed that *BoaZDS* expression varied among different developmental stages and organs. Relative ZDS expression remained stable during germination and seedling stages and rapidly increased at the mature leaf stage. The leaves showed the highest ZDS expression levels compared to the other organs. ZDS expression decreased in all flower tissues during blooming. The fused protein of *BoaZDS* was obtained by prokaryotic expression. Heterologous expression of *BoaZDS* in *Escherichia coli* confirmed that *BoaZDS* encodes a functional ζ -carotene desaturase that increases β -carotene accumulation in *E. coli* cells harboring a β -carotene-producing plasmid. The findings of the present study provide a molecular basis for the elucidation of ZDS gene function in Chinese kale.

Keywords: Chinese kale; *Brassica oleracea* var. *alboglabra* Bailey; carotenoids; ζ -carotene desaturase (ZDS); cloning; gene expression; prokaryotic expression; heterologous expression

1. Introduction

Chinese kale (*Brassica oleracea* var. *alboglabra* Bailey) is a member of the Brassicaceae family that is widely distributed in China and Southeast Asia and occurs at relatively small quantities in Europe, America, and Japan. Chinese kale is generally grown for its bolting stems as common edible parts, which are crispy, tender, and tasty (Sun et al., 2011b, 2012a). Its tender rosette leaves and sprouts are also widely consumed in China (Sun et al., 2011b; Deng et al., 2017). Besides its good flavor, Chinese kale is also a rich source of nutrients, antioxidants, and anticarcinogenic compounds, including vitamin C, glucosinolates, and carotenoids (Sun et al., 2011a, 2011b, 2012a, 2012b).

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 (Pizarro and Stange, 2009; Liu et al., 2015; Nisar et al., 2015). Carotenoids represent a diverse group of pigments that contribute to the red, orange, and yellow colors of various horticultural plants, contributing to their economic value (Tuan et al., 2012; Nisar et al., 2015). Carotenoids participate in various plant physiological processes, including growth, development, and responses to multiple environmental factors. In green tissues, carotenoids act as accessory pigments for the assembly of photosystems and light-harvesting antenna complexes and also have photoprotective functions during photosynthesis (Pizarro and Stange, 2009; Nisar et al., 2015). In non-green tissues, carotenoids confer distinct colors to specialized plastids known as

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