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Molecular characterisation and the light–dark regulation of carotenoid biosynthesis in sprouts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.)

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ABSTRACT

Seven partial-length cDNAs and 1 full-length cDNA that were involved in carotenoid biosynthesis and 2 partial-length cDNAs that encoded carotenoid cleavage dioxygenases were first isolated and characterised in 2 tartary buckwheat cultivars (*Fagopyrum tataricum* Gaertn.), Hokkai T8 and Hokkai T10. They were constitutively expressed at high levels in the leaves and flowers, where carotenoids are mostly distributed. During the seed development of tartary buckwheat, an inverse correlation between transcription level of carotenoid cleavage dioxygenase and carotenoid content was observed. The light-grown sprouts exhibited higher levels of expression of carotenoid biosynthetic genes in T10 and carotenoid content in both T8 and T10 compared to the dark-grown sprouts. The predominant carotenoids in tartary buckwheat were lutein and β-carotene, and very abundant amounts of these carotenoids were found in light-grown sprouts. This study might broaden our understanding of the molecular mechanisms involved in carotenoid biosynthesis and indicates targets for increasing the production of carotenoids in tartary buckwheat.

1. Introduction

Carotenoids are the second most abundant pigment in nature, and the carotenoid family consists of over 700 members (Britton, 1998). Carotenoids fulfill a variety of critical functions in plants, such as harvesting light for photosynthesis, and they constitute the basic structural units of the photosynthesis apparatus (Ledford & Niyogi, 2005). Carotenoids play a main role in furnishing flowers and fruits with the yellow, orange, or red colours that attract animals and facilitate pollination and seed dispersal (Howitt & Pogson, 2006). In addition, the oxidative cleavage of carotenoids produces apocarotenoids, which are plant development signals, antifungal agents, and flavor and aroma contributors to flowers and fruits (Auldridge, McCarty, & Klee, 2006). Carotenoids serve as precursors of vitamin A, which is one of the most important micronutrients that affect the health of humans (Mactier & Weaver, 2005). Furthermore, a diet containing carotenoid-rich vegetables and fruit can protect against some cancers, heart disease, cataracts, and ultraviolet-induced skin damage (Giovannucci, 1999; Mayne, 1996).

Carotenoids are synthesised in plastids by nuclear-encoded enzymes, and the carotenoid biosynthesis pathway in higher plants has been well established (Cunningham & Gantt, 1998). The condensation of 2 geranylgeranyl diphosphates that are catalysed by phytoene synthase (PSY) leads to the first carotenoid, phytoene (Fig. 1). Phytoene is transformed to lycopene by the action of 2 desaturation steps with phytoene desaturase (PDS) and ξ-carotene desaturase (ZDS). The cyclization of lycopene is a key branch point in the pathway, yielding α-carotene that is catalysed by lycopene β-cyclase (LCYB) together with lycopene ε-cyclase (LCYE) and β-carotene that is catalysed by single LCYB. Then, α-carotene and β-carotene are hydroxylated to produce lutein and zeaxanthin, respectively, and catalysed by β-ring carotene hydroxylase (CHXB) and ε-ring carotene hydroxylase (CHXE). Zeaxanthin epoxidase (ZEP) catalyses the epoxidation of zeaxanthin to

Abbreviations: DEPC, diethylpyrocarbonate; DAS, days after sowing; HPLC, high-performance liquid chromatography; GGDP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ξ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHXB, β-ring carotene hydroxylase; CHXE, ε-ring carotene hydroxylase; ZEP, zeaxanthin epoxidase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; ABA, abscisic acid.

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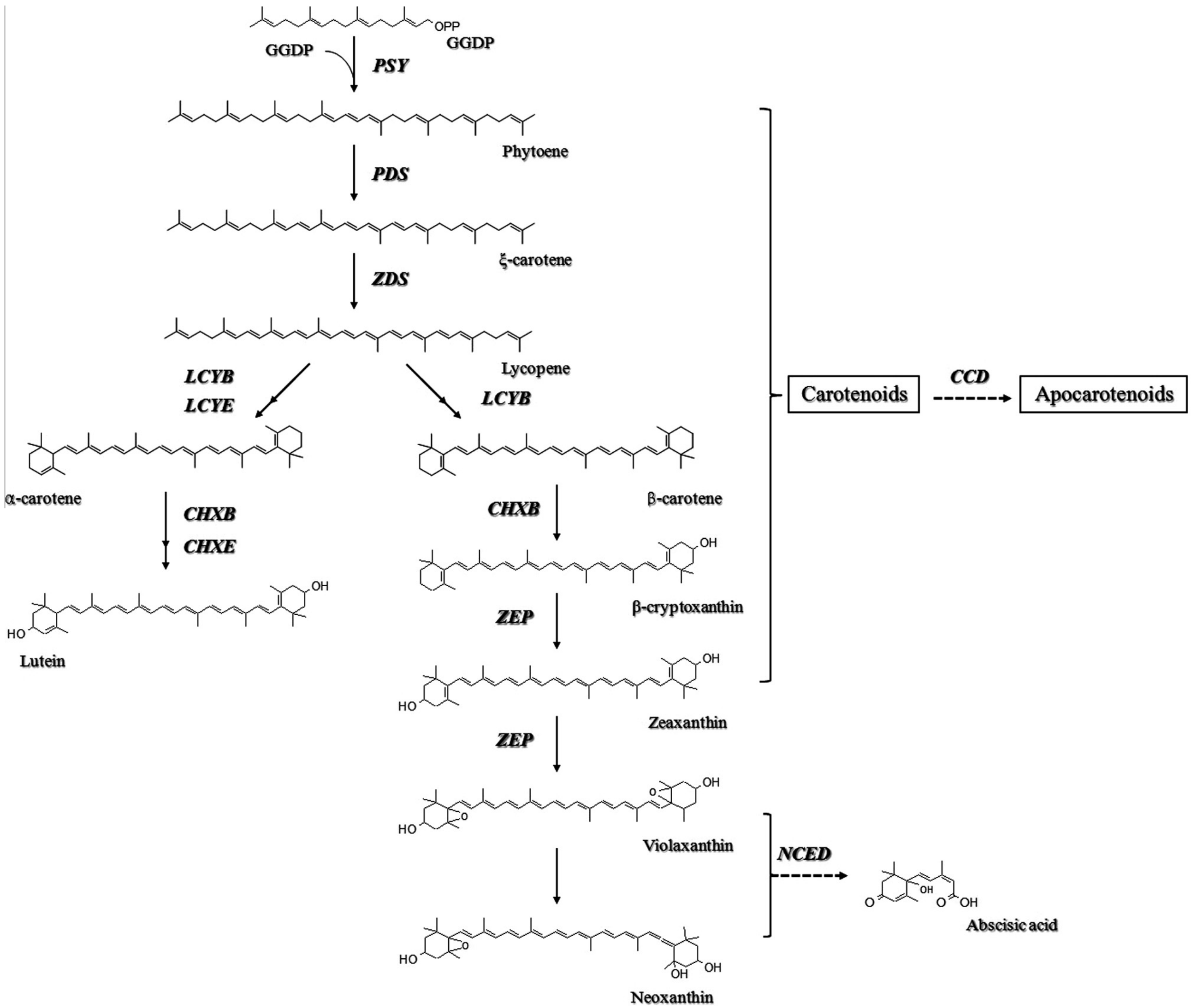

Fig. 1. Carotenoid biosynthetic pathway in plants. GGDP, geranylgeranyl diphosphate; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ξ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHXB, β-ring carotene hydroxylase; CHXE, ε-ring carotene hydroxylase; ZEP, zeaxanthin epoxidase; CCD, carotenoid cleavage dioxygenase; NCED, 9-*cis*-epoxycarotenoid dioxygenase.

produce violaxanthin, which is converted into neoxanthin in the next step. Subsequently, violaxanthin and neoxanthin are used to synthesize the plant hormone abscisic acid (ABA) through oxidative cleavage that is catalysed by 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Schwartz, Tan, gauge, Zeevaart, & McCarty, 1997). Along the pathway, carotenoids can be cleaved into apocarotenoids by carotenoid cleavage dioxygenases (CCD) (Auldridge, McCarty, et al., 2006).

Carotenoid metabolism is a complicated process that is regulated throughout the life cycle of a plant with dynamic changes in content and composition (Cazzonelli & Pogson, 2010). Although plant carotenoids have been widely studied in leaves, flowers, and fruits, little attention has been given to the functions of carotenoids in plant seeds. Generally, seed carotenoids are important for ABA production and seed dormancy, and they also contribute to limiting free radical-induced membrane deterioration and seed ageing (Calucci et al., 2004). To date, the regulation of carotenoid biosynthesis has been investigated in numerous plants, but the mechanisms that control carotenoid accumulation and the expression of carotenoid biosynthesis genes are still unclear. Light has been reported to be an important factor in the regulation of carotenoid biosynthesis in plants (Römer & Fraser, 2005). Light-increased *PSY* mRNA levels result in an increased capacity for the production of carotenoids in *Arabidopsis thaliana* (von Lintig et al., 1997). In contrast, the biosynthesis of carotenoids in *Capsicum annuum* leaves is stalled due to the very low expression of *PSY*, *PDS*, *ZDS*, and *LCYB* in darkness (Simkin, Zhu, Kuntz, & Sandmann, 2003).

Buckwheat that originated in North or East Asia has been considered an important alternative crop in Asia, Europe, North America, South Africa, and Australia for a long time. Two main species of buckwheat are produced around the world. The first is common buckwheat (*Fagopyrum esculentum* Moench), which is usually consumed, whereas, the second, tartary buckwheat (*F. tataricum* Gaertn) is much less common because of its bitter taste. However, tartary buckwheat contains a high content of proteins, fibre, and vitamins B1, B2, and B6 (Bonafaccia, Marocchini, & Kreft, 2003), and it has more flavonoid rutin in comparison to common buckwheat (Suzuki, Honda, & Mukasa, 2005). Numerous previous studies have discussed the anticancer, antidiabetic, and antioxidant properties of tartary buckwheat (Liu, Chen, Yang, & Chiang, 2008; Yao et al., 2008). In addition, seed sprouts of both common and tartary buckwheat are excellent dietary sources of nutrients and phenolic compounds, and they show a good balance of amino acids and minerals (Kim et al., 2008).