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Elevation of lutein content in tomato: A biochemical tug-of-war between lycopene cyclases

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ABSTRACT

Lutein is becoming increasingly important in preventive medicine due to its possible role in maintaining good vision and in preventing age-related maculopathy. Average daily lutein intake in developed countries is often below suggested daily consumption levels, and lutein supplementation could be beneficial. Lutein is also valuable in the food and feed industries and is emerging in nutraceutical and pharmaceutical markets. Currently, lutein is obtained at high cost from marigold petals, and synthesis alternatives are thus desirable. Tomato constitutes a promising starting system for production as it naturally accumulates high levels of lycopene. To develop tomato for lutein synthesis, the tomato Red Setter cultivar was transformed with the tomato lycopene ε-cyclase-encoding gene under the control of a constitutive promoter, and the HighDelta (HD) line, characterised by elevated lutein and δ-carotene content in ripe fruits, was selected. HD was crossed to the transgenic HC line and to RS^{B} with the aim of converting all residual fruit δ-carotene to lutein. Fruits of both crosses were enriched in lutein and presented unusual carotenoid profiles. The unique genetic background of the crosses used in this study permitted an unprecedented analysis of the role and regulation of the lycopene cyclase enzymes in tomato.

A new defined biochemical index, the *relative cyclase activity ratio*, was used to discern post-transcriptional regulation of cyclases, and will help in the study of carotenoid biosynthesis in photosynthetic plant species and particularly in those, like tomato, that have been domesticated for the production of food, feed or useful by-products.

1. Introduction

Lutein is a yellow-coloured carotenoid synthesised in plant organelles (chloroplasts and chromoplasts). Leaves contain the highest levels of lutein since its primary function is as an accessory pigment in photosynthesis, but several fruits and flowers also accumulate lutein, which plays a role in attracting insects and other animals (DellaPenna and Pogson, 2006). Several carotenoids have vitamin A activity, but humans, like other animals, cannot synthesise carotenoids and must obtain them through diet. Although not itself categorised as a vitamin, lutein is of increasing interest as a component of preventive medicine due to evidence suggesting that, in association with zeaxanthin, it plays an important role in maintaining eye health. Lutein and zeaxanthin, collectively referred to as the macular pigment (MP), are concentrated in the human macula (Kijlstra et al., 2012) where they counteract the negative actions of UV-light and reactive oxygen species, thus delaying the onset of age-related macular degeneration and eye diseases (Ma et al., 2012; Karppi et al., 2012; Gao et al., 2011). Observational studies suggest that a diet rich in fruit and green leafy vegetables reduces the risk for many chronic diseases, but scientific substantiation for claims that consuming more lutein through diet or supplementation maintains normal vision is not yet available (EFSA, 2012). Consumption of products containing lutein and other antioxidants has increased steadily in recent years. However, in contrast to the belief that the diet of populations living in developed countries contains a sufficient amount of lutein, it is estimated that daily lutein intake is often below suggested daily consumption levels (Johnson et al., 2010). Lutein intake may be increased to recommended levels by supplementation.

Although lutein is approved in the European Union only as a food additive (EFSA, 2011), new applications are emerging in the cosmetics and personal care industries as well as in the pharmaceutical and nutraceutical markets (Anunciato and da Rocha Filho, 2012; Shegokar and Mitri, 2012). Currently, the most important source of lutein is from the petals of marigold flowers (*Tagetes erecta*). However, the low productivity and the high production costs of

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this system will soon necessitate the use of different species and more efficient agricultural systems to satisfy the growing demand (Fernández-Sevilla et al., 2010). Among crop species, the best candidate to substitute for marigold in lutein production is tomato, as it naturally accumulates substantial amounts of lycopene and is experimentally tractable at the molecular level (D'Ambrosio et al., 2004; Butelli et al., 2008; Huang et al., 2013). However, synthesis of lutein in tomato fruit is hampered by a tightly regulated physiological mechanism that occurs before the start of ripening and involves the down-regulation of the genes encoding the lycopene β- and ε-cyclases (Ronen et al., 1999, 2000; Stigliani et al., 2011). A promising strategy to overcome the physiological block of lutein synthesis in the ripening fruit is metabolic engineering of the pathway, as has been performed in other plants (Römer et al., 2002; Alonso et al., 2011; Sugiyama et al., 2011; Kumar et al., 2012). Our aim in this study was to obtain a tomato line that produced fruits enriched in lutein and other useful carotenoids and had the potential for use in the production of antioxidant-rich food or feed supplements. To reach this objective, we cloned the tomato gene *Lcy-e*, which encodes the lycopene ε-cyclase enzyme necessary for the synthesis of δ-carotene through the cyclization of a ψ end of lycopene (Fig. 1).

Transgenic plants showing high expression levels of *Lcy-e* transgene were selected and characterised for carotenoid content in leaf and fruit. The most promising line, HighDelta, characterised by very high lutein content in leaves and fruit combined with a consistent amount of δ-carotene in ripe fruits, was selected. To complete the synthesis of lutein, HighDelta was crossed to the HighCaro line (D'Ambrosio et al., 2004; Giorio et al., 2007), which contains an *Lcy-b*-based transgene, and to RS^B, a Red Setter isogenic line carrying the *B* (*Cyc-B*) gene (Ronen et al., 2000). The two crosses produced ripe fruits with elevated contents of

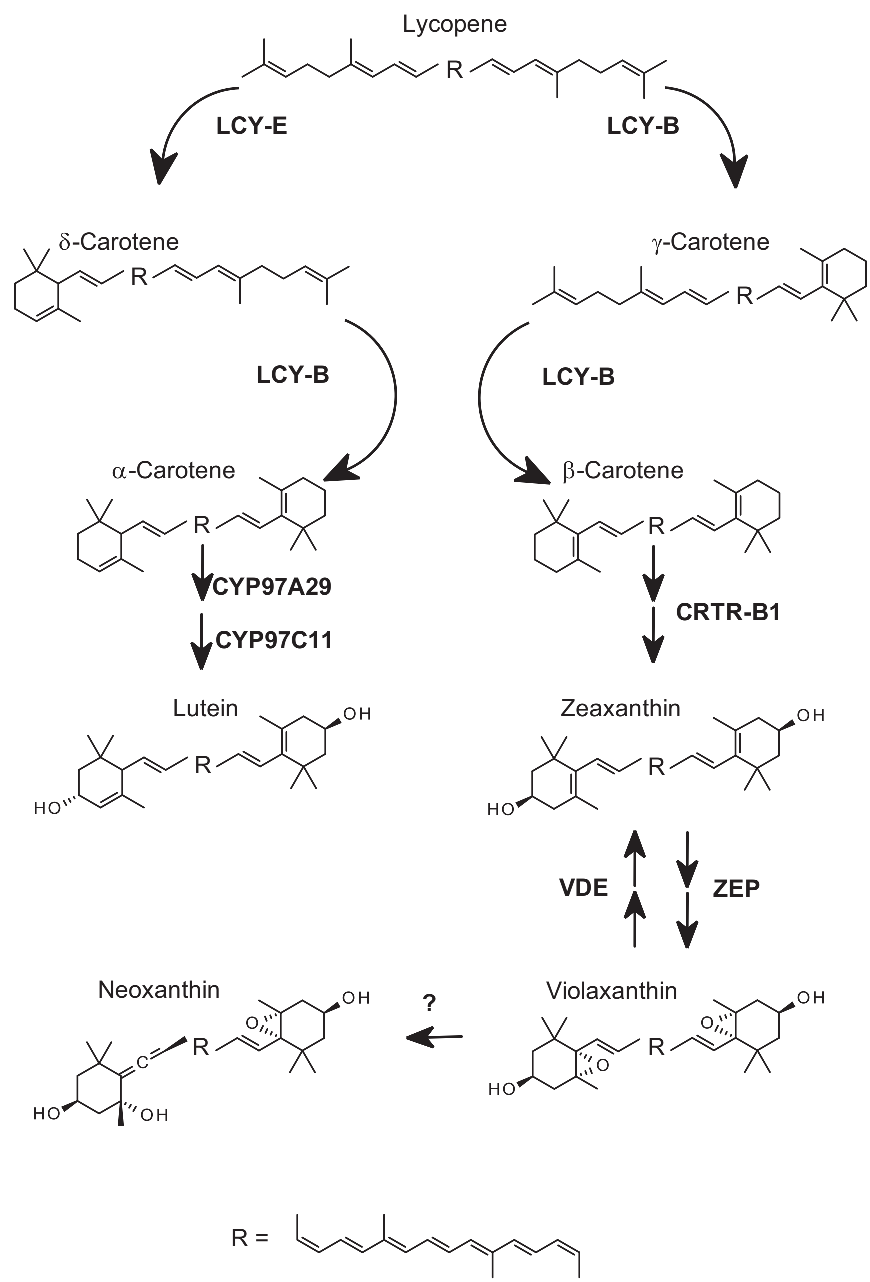

Fig. 1. Xanthophylls pathway in tomato. LCY-E, lycopene ε-cyclase; LCY-B, lycopene β-cyclase; CRTR-B1, β-carotene hydroxylase 1; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; CYP97A29, cytochrome P450-type monooxygenase 97A29; CYP97C11, cytochrome P450-type monooxygenase 97C11. The presence of two arrows indicates that the reaction goes through two successive enzymatic steps.