Available online at www.sciencedirect.com
ScienceDirect
Plant Physiology and Biochemistry 46 (2008) 875–883
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research article

Transgenic carrot plants accumulating ketocarotenoids show tolerance to UV and oxidative stresses

Jayaraman Jayaraj, Zamir K. Punja*

Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada

Received 25 October 2007; accepted 19 May 2008
Available online 6 June 2008

Abstract

Ketocarotenoids are strong antioxidant compounds which accumulate in salmon, shrimp, crustaceans and algae, but are rarely found naturally in higher plants. In this study, we engineered constitutive expression of an algal β-carotene ketolase gene (*bkt*) in carrot plants to produce a number of ketocarotenoids from β-carotene. These included astaxanthin, adonirubin, canthaxanthin, echinenone, adonixanthin and β-cryptoxanthin. Leaves accumulated up to 56 μg/g total ketocarotenoids and contained higher β-carotene levels but lower levels of α-carotene and lutein. The photosynthetic capacity of transgenic plants was not significantly altered by these changes. However, when high-expressing transgenic plants were exposed to UV-B irradiation, they grew significantly better than the wild-type controls. Similarly, leaf tissues exposed to various oxidative stresses including treatment with H_2O_2 and methyl viologen showed less injury and retained higher levels of chlorophyll a + b. Total carotenoid extracts from transgenic leaves had higher antioxidant and free-radical scavenging activity in vitro compared to control leaves. Transgenic tissues also accumulated lower amounts of H_2O_2 following exposure to oxidative stresses, suggesting that free radical and reactive oxygen species were quenched by the ketocarotenoids.

Keywords: β-Carotene ketolase; Oxidative stress tolerance; UV-B tolerance; *Daucus carota*

1. Introduction

In higher plants and algae, most environmental stresses, including temperature extremes, drought and salt stress, high light and UV exposure, and various oxidant chemicals, cause an overproduction of reactive oxygen species (ROS) [1]. The steady-state level of ROS intermediates can be used by plants to monitor their intercellular level of stress and for cell signaling. However, this level has to be kept under strict control because an over-accumulation of reactive oxygen intermediates can result in cell death [2], due to membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage [1]. Damage to plants from accumulation of ROS and free radicals resulting from various environmental stresses is a major cause of loss in crop productivity worldwide and alleviation of these stresses can ultimately lead to enhanced crop yield [1].

Carotenoids are relatively photostable pigments and antioxidants produced by plants, bacteria and cyanobacteria [3] which quench free radicals, thereby protecting cells and tissues from oxidative damage [4]. Over 600 naturally occurring carotenoids are known, with β-carotene being the most abundant antioxidant compound [5,6]. Oxidative stresses can also upregulate the synthesis and accumulation of carotenoids in plants [7].

Oxygenated carotenoids (ketocarotenoids) are found in the flesh of salmon, shells of shrimp and other crustaceans, and feathers of birds such as flamingoes and quails [8].

They occur rarely in higher plants [9], although they are abundantly present in the red algae, *Haematococcus pluvialis* [10]. The ornamental flowering plant *Adonis aestivalis* accumulates ketocarotenoids [11], and ketocarotenoids such as capsanthin and capsorubin are found in *Capsicum annuum* [12]. Research has demonstrated that ketocarotenoids such

* Corresponding author. Tel.: +1 778 782 4471; fax: +1 778 782 3496.
E-mail addresses: jaya@sfu.ca (J. Jayaraj), punja@sfu.ca (Z.K. Punja).

0981-9428/$ - see front matter © 2008 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2008.05.015

as astaxanthin have high antioxidant potential (surpassing that of lycopene, β-carotene and α-tocopherol) [13]. In humans, these compounds may prevent cardiovascular disease, boost the immune system, provide antibacterial and anticancer properties and prevent cataracts and tissue damage from ultraviolet radiation [8]. In algae, ketocarotenoids were shown to accumulate in higher quantities in lipid vesicles in response to various stress conditions, including nutrient deprivation and high irradiation levels, suggesting a role in providing tolerance to stress conditions [14]. In plants, because of their rare occurrence, the role of ketocarotenoids in enhancing antioxidant activity has not been previously demonstrated.

There are previous studies which have demonstrated enhanced tolerance to UV in plants over-expressing a β-carotene hydroxylase gene in Arabidopsis [15] and tobacco [16], which resulted in an over-accumulation of zeaxanthin (a non-ketocarotenoid compound). The cyanobacterium *Synechoccus* transformed with an *H. pluvialis* β-carotene ketolase accumulated canthaxanthin (a ketocarotenoid), and the transformed strain was tolerant to UV-B and strong light radiation [17]. Similar studies to demonstrate the role of ketocarotenoids in enhancing plant stress tolerance are lacking. The objective of this study was to evaluate the tolerance of ketocarotenoid-accumulating transgenic carrot plants to UV-B and other induced oxidative stresses. The antioxidant and free-radical scavenging activity of ketocarotenoid-containing plant extracts was also determined.

2. Results and discussion

2.1. Generation of transgenic plants and molecular analysis

Following *Agrobacterium*-mediated transformation, 80 carrot plant lines, each derived from different explants, were generated and grown in a greenhouse and screened for resistance to Liberty herbicide. A total of 65 plant lines showed complete resistance to Liberty at 0.2%. All these lines were tested by PCR and found to be positive for both *bar* and *bkt* genes (Fig. 1a and b). Southern analysis for the *bkt* gene confirmed the presence of the gene (Fig. 1c). Northern analysis showed accumulation of transcripts in leaves at high levels in 33 lines (Fig. 1d) with the remainder of the lines having very low or no expression. Western analysis confirmed the presence of recombinant ketolase protein in the leaves at varying levels (Fig. 1e).

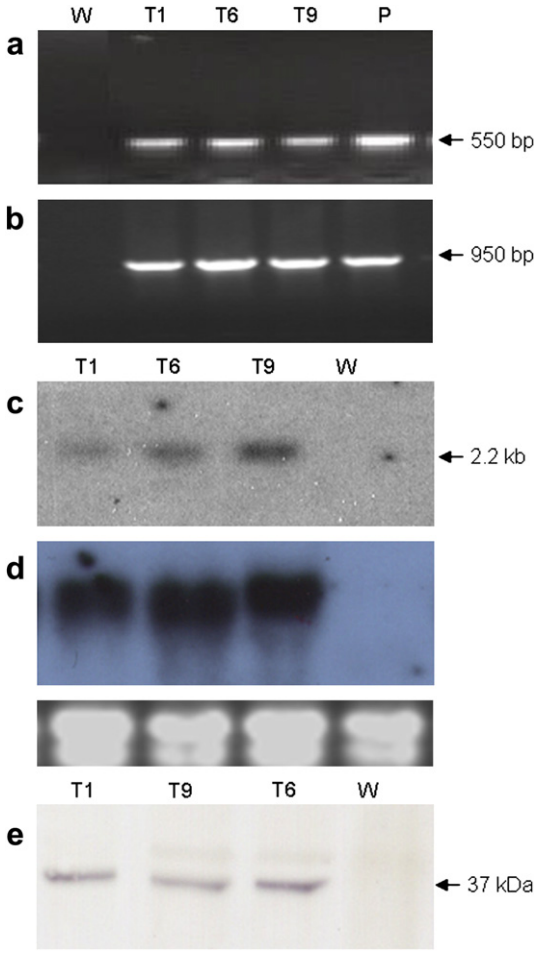

Fig. 1. Molecular analyses of transgenic carrot plants; (a) PCR for *bar* gene; 200 ng of plant genomic DNA was used for each reaction; numbers indicate plant lines; P, plasmid positive control; arrow indicates amplicon size. (b) PCR for *bkt* gene. (c) Southern blot for *bkt* gene; 10 μg of genomic DNA was digested with *BamH*I and probed with *bkt* cDNA fragment. (d) Northern blot for *bkt* gene; 10 μg of total RNA was probed with *bkt* cDNA fragment; bottom panel – RNA loading. (e) Western blot for BKT protein; 50 μg of total proteins were resolved in a 12% or 15% SDS–PAGE, blotted and probed with an anti-BKT-peptide antiserum.

2.2. Carotenoid profile in leaves

Carotenoids from transgenic and wild-type leaves were extracted and compared. Wild-type leaves contained α-carotene, β-carotene, lutein and zeaxanthin along with some unidentified carotenoids. Transgenic leaves contained all of the carotenoids found in wild-type plants, as well as new ketocarotenoids, including astaxanthin, adonixanthin, adonirubin, canthaxanthin, β-cryptoxanthin and echinenone, which were absent in wild-type tissues (Fig. 2). Carotenoid levels in three high-expressing transgenic lines and the mean of three wild-type plants is presented in Table 1. While there was some minor variation in the levels between the three transgenic lines (which was not statistically different), the trends were similar. As an additional control, low or nil-expressing lines were analyzed and found to contain negligible to non-detectable levels of these ketocarotenoids (data not shown). HPLC and mass spectrometry analyses confirmed the chemical identity of the unique carotenoids (data not shown).

Astaxanthin was present at the highest level (29.6–34.7 μg g^{-1} fresh weight). In transgenic leaves, the lutein content was reduced by 34% compared to the wild-type. Significantly lower amounts of zeaxanthin (by 20%) and α-carotene (by 34%) were also observed in transgenic leaves. In contrast, the β-carotene content was significantly higher (by 24%) in transgenic leaves compared to wild-type. Further, the total carotenoid content was slightly higher (by 9.3%) in