Environmental and Experimental Botany 75 (2012) 1–8

Contents lists available at SciVerse ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Carotenoid content, leaf gas-exchange, and non-photochemical quenching in transgenic tomato overexpressing the β-carotene hydroxylase 2 gene (CrtR-b2)

Pasquale Giorio[a,*], Giovanni Giorio[b], Carmela R. Guadagno[c], Francesco Cellini[b], Lucia A. Stigliani[b], Caterina D'Ambrosio[b]

[a] Institute for Agricultural and Forestry Systems in the Mediterranean, National Research Council of Italy, Via Patacca, 85, 80056 Ercolano, Naples, Italy
[b] Metapontum Agrobios, S.r.l., S.S. Jonica 106, Km 448,2–75010 Metaponto, Matera, Italy
[c] Department of Structural and Functional Biology, University of Naples Federico II, Via Cinthia, 4-80100 Naples, Italy

ARTICLE INFO

Article history:
Received 11 April 2011
Accepted 22 August 2011

Keywords:
Tomato
β-Carotene hydroxylase
Carotenoids
Xanthophyll cycle
Chlorophyll *a* fluorescence
Non-photochemical quenching

ABSTRACT

Non-photochemical quenching (NPQ) of chlorophyll *a* fluorescence and leaf gas-exchange were investigated in relation to the chlorophyll and carotenoid content, and the xanthophyll cycles in wild type tomato (*Solanum lycopersicum*, L. *cv* Red Setter (RS)) and in two transgenic lines (UO and UU) over-expressing β-carotene hydroxylase. Potted plants were grown in a glasshouse under low light (LL, 100 μmol m^{-2} s^{-1}) or high light (HL, 300 μmol m^{-2} s^{-1}). The maximum quantum efficiency of photosystems II (PSII) photochemistry in dark-adapted leaves (F_v/F_m) was higher than 0.82 in all treatments while photosynthetic CO_2 assimilation (A) was higher than 14 μmol m^{-2} s^{-1}, and stomatal conductance (g_s) higher than 0.4 mol m^{-2} s^{-1} in HL plants, indicating no effects induced by the genetic modification. Chlorophyll content and composition changed little, whereas transgenic plants had up to 47% higher total carotenoid content than wild type plants. Violaxanthin was the most abundant carotenoid in transgenic plants, with more than 2-fold higher content than the average 0.586 mg g^{-1} found in RS plants. Transgenic plants had similar light-induced steady-state NPQ compared to wild type plants, but had slower dark relaxation because of the decreased de-epoxydation state index due to the higher violaxanthin accumulation, despite the higher zeaxanthin content.

1. Introduction

Under high irradiance, the absorbed light energy exceeds the saturation point of photosynthesis and the photosynthetic apparatus can experience a potentially harmful condition (Foyer et al., 1994; Oguchi et al., 2009). As a result, reactive species of oxygen (ROS) may be generated (Blankeship, 1998), with a consequent photooxidative damage (Macpherson et al., 1993; Telfer et al., 1994; Niyogi, 1999). A line of defense against ROS reactions is developed by carotenoids (Cars). These molecules are able to harvest the excitation energy and they contemporary play a fundamental role in the photoprotective mechanism, quenching the excited state of chlorophylls (Krinsky, 1978; Blankeship, 1998; Bassi and Caffarri, 2000; Kalituho et al., 2007b). Improved stress tolerance was found in rice after exogenous application of β-carotene (β-car) (Yang et al., 2002). On the contrary, a very low viability of tomato *ghost* mutants has been demonstrated and it seems to be caused by the inhibition of the carotenoid biosynthetic pathway (Scolnik et al., 1987; Sandmann and Böger, 1989). In addition, carotenoids are precursors of vitamin A, and their beneficial action in the prevention of cancers and other mammalian diseases is widely recognised (Krinsky, 1989).

As soon as the absorbed energy by plant pigments exceeds the requirement for photochemical activity, a fast mechanism of heat dissipation is triggered, in order to prevent ROS production (Demmig-Adams and Adams, 1992; Szabo et al., 2005; Eberhard et al., 2008). This mechanism is known as non-photochemical quenching (NPQ) of chlorophyll fluorescence because it competes with photochemistry to quench the little energy that the excited chlorophyll *a* molecules can re-emit as fluorescence (Horton et al., 1994; Maxwell and Johnson, 2000; Müller et al., 2001; Demmig-Adams, 2003; Holt et al., 2004; Krause and Jahns, 2003; Baker, 2008). NPQ is a composite of three different components, each of which is characterised by a peculiar kinetic behavior. The major and quickly reversible component of NPQ is the energy-dependent quenching (qE) that relaxes in darkness within 2–3 min (Niyogi et al., 2005; Pascal et al., 2005).

The intermediate quenching component is named qT for its relation with the state transitions, a mechanism which balances the

* Corresponding author. Fax: +39 0817718045.
E-mail address: pasquale.giorio@cnr.it (P. Giorio).

0098-8472/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2011.08.009

excitation pressure between PSII and PSI, through a reversible phosphorylation of LHCII proteins (Haldrup et al., 2001). Today, qT is believed to be associated with the conversion of Vio into Zea and it is now designated as qZ (Nilkens et al., 2010). The induction and relaxation time of qZ coincide with the formation and re-epoxidation of Zea within the 10–15 min time range that was before attributed to qT. Another component of NPQ is qI. This component is commonly associated with the damage of the D1 protein that leads to photoinhibition and lower photosynthetic capacity (Aro et al., 1993), but also Zea seems to be involved in the triggering of this mechanism (Jahns & Miehe, 1996; Thiele et al., 1996; Verhoeven et al., 1996). qI relaxation takes more than 20 min (Walters and Horton, 1993; Lee et al., 2001; Matsubara and Chow, 2004).

Since the 1980s, the enzymatic inter-conversion of zeaxanthin (Zea) via the intermediate anteraxanthin into violaxanthin (Vio) in the xanthophyll cycle (Yamamoto et al., 1962) has been linked to the safe dissipation of excess excitation energy as heat within the PSII antenna (Demmig et al., 1987; Demmig-Adams and Adams, 1996; Ruban and Horton, 1999). However, also lutein (Lut) (Ruban et al., 2007) can thermally dissipate the excess excitation energy (Ruban, 2009). These xanthophylls, which are differently bound to light-harvesting complex II (LHCII) (Bassi et al., 1993; Croce et al., 1999; Morosinotto et al., 2002; Passarini et al., 2009), are thought to act through different molecular quenching mechanisms whose relative importance is still uncertain (Ruban, 2009). There are three current models for the qE molecular-mechanism in higher plants. They all agree on the requirement of the generation of the Δ-pH gradient across thylakoid membranes and the involvement of a protein of the light-harvesting complex (LHCII), PsbS (Ruban et al., 2007; Johnson et al., 2009).

The first model proposes that high light condition causes a decrement in the pH value of thylakoid lumen and the protonation of PsbS with the contemporary production of Zea via the xanthophyll cycle (Li et al., 2002a; Niyogi et al., 1997). The protonated PsbS induces a conformational change in antenna proteins and the formation of a quenching complex (Li et al., 2002b). In this model, the de-excitation happens via charge separation and subsequent recombination in a Chl–Zea complex. The aggregation of LHCII proteins plays a central role in the second model for qE. It was observed that oligomerization of LHCII trimers leads to quenching of chlorophyll fluorescence (Pascal et al., 2005). Raman spectroscopy indicated this quenching is accompanied by a twist in a neoxanthin (Neo) molecule (Pascal et al., 2005). A conformational change that brings one or two chlorophylls together which can then transfer the excess energy to a nearby Lut in the major antennas (Ruban et al., 2007). In this mechanism Zea plays an allosteric role. A third possible mechanism is the Chl–Chl charge transfer (Miloslavina et al., 2008; Müller et al., 2010). According to this model, the quenching mechanism arises from a Chl dimer that undergoes charge transfer and subsequent emission to the ground state, with no energy transfer to xanthophylls. Consequently, LHCII antennas are detached from the PSII cores in high light and most probably quenched by this Chl–Chl charge transfer mechanism as reported in Holzwarth et al. (2009). PsbS is required and believed to allow for the detachment of the LHCII from the supercomplex.

The characterisation of plant and green algae mutants is undoubtedly the most powerful approach adopted by many research groups to unveil the underlying mechanisms of NPQ (e.g. Niyogi et al., 1998; Pogson et al., 1998; Baroli and Niyogi, 2000; Niyogi et al., 2004; Dall'Osto et al., 2007; Kalituho et al., 2007a; Johnson et al., 2009; Ruban, 2009). However, plant metabolic engineering has also been fruitfully used to investigate the xanthophylls role in NPQ mechanisms. Genetically modified *Arabidopsis* with over-expressed β-car hydroxylase acquired an enhanced xanthophyll cycle and better light stress tolerance (e.g. Davison et al., 2002; Johnson et al., 2007, 2008). Conversely, the over-expression of Zea epoxydase gene in tomato enhanced the PSII photoinhibition sensitivity to high light and chilling stress (Wang et al., 2008).

Transgenic tomato (*Solanum lycopersicum* L. *cv* Red Setter) lines with enriched carotenoid content in leaves, flowers and fruits have been obtained at Metapontum Agrobios (Metaponto, MT, Italy) through the over-expression of the tomato β-car hydroxylase 2 (CrtR-b2) gene driven by the constitutive 35S CaMV promoter (D'Ambrosio et al., 2011). In wild type tomato, the CrtR-b2 gene as well as its paralogous CrtR-b1 encodes a carotenoid-specific hydroxylase belonging to the non-heme diiron beta-ring group (Galpaz et al., 2006). These enzymes are involved in the synthesis of xanthophylls, mainly Zea, through the hydroxylation of the beta-ionone ring. Following transformation of tomato with a transgene carrying the CrtRb2 cDNA under the control of the 35S promoter, it was possible to select transgenic lines over-expressing the CrtRb2 transgene in all tissues, particularly in leaves where the Vio content was strongly increased (D'Ambrosio et al., 2011). This paper examines the hemizygous UO line and the homozygous UU line for the transgene encoding the CRTRB2 β-car hydroxylase, which is involved in the hydroxylation of the β-car ionone ring. Increased leaf xanthophyll content through genetic engineering would potentially improve the stress tolerance of these lines, as found in *Arabidopsis* (Davison et al., 2002). We tested the hypothesis that the increased xanthophyll content would improve the mechanisms of thermal dissipation, through the analysis of NPQ induction and relaxation for the transgenic UO and UU tomato lines compared to the control Red Setter (RS). We also report the physiological characterisation of the three genotypes in terms of maximum efficiency of photosystems II (PSII) photochemistry (F_v/F_m), gas-exchange and leaf chlorophyll and carotenoid contents.

2. Materials and methods

2.1. Plant material and experimental design

The experiment was carried out in the glasshouse facilities at Metapontum Agrobios (Metaponto, MT, Italy). Seeds of the three tomato genotypes (transgenic homozygous (UU) and hemizygous (UO) lines, and control RS) were germinated in plastic trays containing a compost made of equal volumes of a clay soil and peat (Flora Gard Sub Professional, Type S 0.5, Floragard, Villa Lagarina, TN, Italy). Seedlings were grown to the 2–3 true leaf stage at 24 °C for 8 h light/16 h dark, with a photosynthetic photon flux density (PFD) of 150 μmol photons m^{-2} s^{-1}. Plants were then transplanted into 10 litre pots, one plant per pot, filled with clay soil and grown in a heated glasshouse with either low-light (LL; 100 μmol photons m^{-2} s^{-1}) or high-light (HL; 300 μmol photons m^{-2} s^{-1}). Four plants from each of the three genotypes were allocated to each experiment according to a randomized block design. Plants were grown using standard cultural practices at a daily average temperature of 26 °C with artificial light provided by 400 W metal halide lamps (HPI-T 400 W, Philips) with a 16 h light/8 h dark photoperiod. The transplanted plants were grown for 3 weeks before analysis.

2.2. Light adapted leaf gas-exchange

Measurements were taken on one leaf per plant for 4 plants per treatment. Light-adapted leaf CO_2 assimilation (A, μmol m^{-2} s^{-1}) and stomatal conductance to water vapour (g_s, mol m^{-2} s^{-1}) were measured by means of a portable photosynthesis system (Li-6400, LiCor, Lincoln, NE, U.S.A.). Actinic light was provided by an artificial red and blue LED source with 470 and 630 nm emissions, respectively. The light source was set at a saturating PFD (10% blue light) of 1800 μmol m^{-2} s^{-1} for HL plants and 750 μmol m^{-2} s^{-1} for LL