Plant Physiology and Biochemistry 90 (2015) 23–31

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Ultraviolet-B protection of ascorbate and tocopherol in plants related with their function on the stability on carotenoid and phenylpropanoid compounds

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ARTICLE INFO

Article history:
Received 4 December 2014
Accepted 26 February 2015
Available online 27 February 2015

Keywords:
UV-B radiation
Ascorbate
Vitamin E
Carotenoid
Phenylpropanoid
Oxidative stress

ABSTRACT

Ascorbate and tocopherol are important hydrophilic or lipophilic antioxidants in plants, while their crucial roles in the antioxidant defense system under ultraviolet B radiation were not well understood. The mutants of *Arabidopsis thaliana* deficient in ascorbate (*vtc1* and *vtc2*) or tocopherol (*vte1*) were used to analyze their physiological, biochemical and metabolic change in responses to Ultraviolet B radiation. Results showed that loss of either ascorbate or tocopherol caused reduction in phenylpropanoid and flavonol glycosides compounds, as well as reduction in superoxide dismutase activity and total cellular antioxidant capacity. This ultimately led to higher oxidative stress as well as lower levels of photosynthetic pigments (carotenoid and chlorophyll) and CO_2 assimilation rate in the *vtc1*, *vtc2*, and *vte1* mutants than the wild type under UV-B radiation, besides unstable early light-induced protein (ELIP1) in those mutants. On the other hand, the loss of tocopherol in *vte1* mutants was compensated by the increase of zeaxanthin and anthocyanin contents, which armed *vte1* mutants with higher heat dissipation capacity in PS II and higher antioxidative capacity than *vtc* mutants. Consequently the tolerance to UV-B radiation were much higher in *vte1* mutant than in *vtc* mutants, furthermore, PS II function and light harvesting protein (LHCb1) abundance were reduced only in ascorbate-deficient mutant relative to wild type. Our results suggested that the ascorbate and tocopherol provided not only direct protective function against UV-B radiation but also indirect effects by influencing other protective system, in particular by affecting the stability of various carotenoid and phenylpropanoid compounds.

1. Introduction

The intensity of solar ultraviolet-B radiation (UV–B; 290–315 nm) reaching the ground vary with altitude, latitude, season, and surface atmosphere (Rajakumar et al., 2007; Yao et al., 2008). Although UV-B irradiance comprises only 1.5% of the total solar radiation energy, it serves as a major potential on photoinhibition, and could impose direct and indirect damage on cellular macromolecules, including nucleic acids, lipids, and proteins (Hollósy, 2002). However, plants in nature can grow under quite diverse UV-B environments, and is seldom damaged by UV-B, depending on effective protection mechanisms (Wolf et al., 2010). A common protective response against UV-B is to elicit antioxidant enzymes and non-enzyme antioxidant compounds, of the latter including the water- or the lipid-soluble antioxidants, such as ascorbate and tocopherol.

Substantial variations occur in ascorbate levels under different environmental stresses. Acute UV-B exposure leads to net oxidation of ascorbate, while chronic UV-B exposure increases the ascorbate pools (Jansen et al., 2008). Ascorbate plays a crucial role in complex antioxidation processes (Conklin, 2001). Previous studies have shown that chronic UV-B exposure caused leaf chlorosis in the Arabidopsis *vtc1* mutant due to ascorbate deficiency, accompanying with higher oxidative damage and insufficient activity in antioxidative enzymes (Gao and Zhang, 2008). Ascorbate abundance in

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http://dx.doi.org/10.1016/j.plaphy.2015.02.021
0981-9428/

plants could influence other protective mechanisms. Recent studies demonstrated that the ascorbate-deficient mutants accumulated less anthocyanin, more glutathione, more limited violaxanthin de-epoxidase activity and lower capacity in nonphotochemical energy dissipation (NPQ) of photosystem II than wild-type during high light acclimation (Page et al., 2012; Foyer and Noctor, 2011). But the function of ascorbate under UV-B radiation is not well-understood.

Tocopherol belongs to the Vitamin E class of lipid soluble antioxidants that are essential for human nutrition and uptake of plant-derived oils and fats (Szarka et al., 2012). α-tocopherol is the major vitamin E compound in plant leaves, and the α-tocopherol levels change differentially in response to environmental constraints. Early studies showed that short-term strong UV-B exposure decreased α-tocopherol levels in plants, but long-term UV-acclimation increased tocopherol levels (Delong and Steffen, 1997). α-tocopherol is synthesized in the inner envelope of chloroplasts and accumulates in all chloroplast membranes. It deactivates photosynthesis-derived reactive oxygen species (mainly $^1O_2$ and OH·), and scavenges lipid peroxyl radicals against the propagation of lipid peroxidation in thylakoid membranes, protecting the photosynthetic apparatus. Li et al. (2012) observed that the over-accumulation of tocopherols in the engineered *Chlamydomonas reinhardtii* strains enhanced resistance to high light stress and significantly improved photosystem II efficiency. Havaux et al. (2005) reported that the leaf discs of two vitamin E deficiency mutants showed bleaching phenotype and lipid photodestruction as compared to wild type Arabidopsis, when exposed to combined stresses of high light and low temperature. Despite a fast progress in tocopherol function exploration, its role in response to UV-B radiation was not clear yet.

On the other hand, the interplay between the water- and the lipid-soluble antioxidants is important for plant tolerance to environmental stresses (Szarka et al., 2012). Deficiency in any antioxidants, such as ascorbate, tocopherol or glutathione, would lead to increases of alternative antioxidants simultaneously, which could afford compensatory protection for the photosynthetic apparatus. In vitro experiments showed that the tocopherol-mediated protection against lipid peroxidation is strongly enhanced by the presence of ascorbate and glutathione (Kanwischer et al., 2005). Phenylpropanoid and carotenoid pigments are also important water- and lipid-soluble antioxidants in plant leaves, respectively, and it is merited to understand their role interacting with or compensating for ascorbate or tocopherol against UV-B radiation.

In the present study, ascorbate or tocopherol-deficient mutants and their wild-type Arabidopsis plants were exposed to UV-B radiation to analyze the photosynthesis performance, antioxidative responses, and changes of different carotenoid and phenylpropanoid compounds. Our objective was to determine the protective role of ascorbate and tocopherol in plant leaves under UV-B radiation as well as their influence on different carotenoid and phenylpropanoid compounds.

2. Material and method

2.1. Plant materials and growth conditions

Arabidopsis wild type (Columbia ecotype, Col), *vtc1*, *vtc2* and *vte1* mutants were grown on sand incubated with Hoagland nutritive solution under controlled conditions (260 μmol m^{-2} s^{-1} photosynthetically active radiation light, 16/8 h in light/dark cycles with 22/18 °C, and relative humidity at 60%). Seeds of different mutants were obtained from the Nottingham Arabidopsis Seeds Centre. The *vtc1* and *vtc2* separately carries a mutation in the genes encoding GDP-mannose pyrophosphorylase and GDP-L-galactose phosphorylase that are involved in ascorbate synthesis, and therefore these plants contains less than 35% of wild-type amounts of ascorbate (Conklin et al., 1999; Linster et al., 2007). The *vte1* mutant is defective in tocopherol cyclase activity and deficient in all tocopherols (Porfirova et al., 2002).

Six-week-old plants were used for the experiments. Plants received UV-B radiation intensity about 0.09 W m^{-2} s^{-1} (280–315 nm; Beijing Electronic Resource Institute, Beijing, China) or not (CK) for 8 h each day for five days under the same growth conditioned as above-mentioned. At the end of the experiment, the fully-expanded leaves of WT and mutant plants were harvested, weighed, immediately frozen in liquid nitrogen, and stored at −80 °C prior to analysis.

2.2. Chlorophyll fluorescence and photosynthetic rate measurements

Photochemical efficiency was estimated at 12:00 every day after four hours of UV-B treatment for the upper surface of the leaves via chlorophyll a fluorescence of photosystem II (PS II) using a modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany) as described by Havaux and Kloppstech (2001). The maximum photochemical efficiency of Photosystem II (PSII) was calculated as Fv/Fm = (Fm − F0)/Fm. Fv/Fm was measured in UV-B stressed leaf discs after 30 min adaptation to darkness. The actual PSII efficiency (ΦPSII) was calculated using the equation: $\Phi PSII = (F'm - Fs)/F'm$. A saturating light pulse was applied to dark-adapted plants and subsequently the photosynthetic photon flux density (PPFD) stepwise increased to record light-response NPQ curves. NPQ was calculated using the equation: NPQ = Fm/F′m-1 (Bilger and Björkman, 1990). The photosynthetic rate (CO_2 assimilation rate) was also measured by portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln, NE) between 9: 00 and 11: 00 h every day.

2.3. Determination of enzyme activities

Plant tissues were ground in mortar with liquid nitrogen, and extracted by ice-cold buffer (100 mM KH_2PO_4/K_2HPO_4, pH 7.8; 1% Triton X-100; 5 mM ascorbate; 1% PVP). Protein concentrations were determined according to Bradford method (1976) with bovine serum albumin (BSA) as a standard. The superoxide dismutase (SOD) activity was measured spectrophotometrically according to the method of Tanaka and Suigahara (1980). The catalase (CAT) activity was determined by measuring H_2O_2 consumption according to the method of Aebi (1983). Guaiacol peroxidase (Gu-POD) activity was measured at 436 nm by the method of Nakano and Asada (1981).

2.4. Determination of thiobarbituric acid reacting substances (TBARS), ascorbate and H_2O_2 contents

Samples were ground in 5% trichloroacetic acid (TCA), and then centrifuged at 16, 000 × *g* at 4 °C. The supernatant was used to determine the content of thiobarbituric acid reacting substances (TBARS), a lipid peroxidation index, or neutralized to pH6 or pH7 with 4N KOH for determination of ascorbate (Asa) or H_2O_2 content. The TBARS contents were determined according to the method of Dhindsa and Matowe (1981). H_2O_2 was determined according to the method of Okuda et al. (1991) after the supernatant was cleaned by anion exchange resin (AG-1, Bio-Rad). AsA and dehydroascorbate (DHA) were determined by a method of Foyer et al. (1983).

2.5. Chlorophylls and carotenoids measurements

For photosynthetic pigment analyses, leaf pigments were