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Overexpression of a tomato carotenoid ε-hydroxylase gene (*SlLUT1*) improved the drought tolerance of transgenic tobacco

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ABSTRACT

Drought stress is a considerable environmental factor that restrains photosynthesis. Lutein, the most prolific carotenoid in plant photosynthetic tissues, plays vital roles in the light-harvesting complexes. However, its biological functions under abiotic stresses remain unclear. In our research, transgenic tobacco plants were utilized to investigate the function of the tomato chloroplast-targeted carotenoid epsilon-ring hydroxylase (SlLUT1) in drought stress tolerance. The analysis of *SlLUT1*-pro-*LUC* and qRT-PCR showed that drought stress induced *SlLUT1* expression. Transgenic tobacco plants exhibit higher lutein content than wild-type (WT) tobacco. Under drought stress, transgenic plants overexpressing *SlLUT1* showed better growth performance, higher chlorophyll and relative water contents and more intact chloroplast and PSII supercomplex structures than WT tobacco. The Fv/Fm, Pn, NPQ, and content of D1 protein in transgenic plants were higher than those in WT plants under drought stress. The accumulation of H_2O_2 and $O_2\cdot^-$ decreased in transgenic tobacco plants. Moreover, transgenic plants exhibited lower MDA accumulation and REL. These results indicate that overexpression of *SlLUT1* enhances tolerance to drought stress by maintaining photosynthesis and scavenging ROS in transgenic tobacco.

1. Introduction

Plants live in constantly changing conditions and frequently suffer different types of abiotic stress, such as drought, light, salinity, heat and cold. These stresses seriously inhibit plant growth rates and consequently decrease productivity. Drought stress is an important abiotic stress that limits plant growth. It can cause stomatal closure and damage chloroplast structure. In addition, excess light energy, which is associated with drought stress, reduces photosynthetic efficiency, resulting in photoinhibition and even photooxidation (Pastenes et al., 2005). D1 protein, a significant subunit of the photosystem II (PSII) reaction center complex, is also destroyed by excess light energy. However, the damaged D1 protein can be degraded, re-synthesized, and reassembled into the PSII complex (Zhou et al., 2013). This cycle is called D1 cycle, and it contributes to the repair of damaged PSII (Kato and Sakamoto, 2009; Nishiyama et al., 2006).

Drought stress also induces the congestion of reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2) as well as superoxide anion radical ($O_2\cdot^-$). ROS ultimately exert oxidative stress on plants. Plant chloroplasts possess two pathways for ROS congestion: (1) electron transfer to molecular oxygen at the acceptor side of photosystem I (PSI) or at PSII (Ananyev et al., 1994), resulting in the congestion of the $O_2\cdot^-$ in subsequent reactions (Apel and Hirt, 2004); and (2) energy transformation from triplet chlorophyll ($^3Chl^*$) to molecular oxygen, causing the congestion of singlet oxygen ($^1O_2$) (Krieger-Liszkay, 2005; Triantaphylides and Havaux, 2009). Photosynthetic organisms have developed several defense mechanisms to mitigate ROS congestion or detoxify formed ROS (Li et al., 2009). Furthermore, ROS scavenging systems involve some antioxidases, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), to protect plants from ROS damage. In the PSII reaction center, carotenoids play a considerable role in the deactivation of $^3Chl^*$ and $^1O_2$.

Abbreviations: APX, ascorbate peroxidase; BN-PAGE, blue native polyacrylamide gel electrophoresis; CAT, catalase; DAB, 3,3-diaminobenzidine; DTT, DL-dithiothreitol; HPLC, high-performance liquid chromatography; H_2O_2, hydrogen peroxide; LHC II, light-harvesting complex II; LUC, luciferase; MDA, malondialdehyde; Fv, fluorescence; Fm, maximal fluorescence; Fo, minimal fluorescence; Fv/Fm, maximum photochemical efficiency of photosystem II; NBT, nitroblue tetrazolium; NPQ, non-photochemical quenching; $O_2\cdot^-$, superoxide anion radical; PEG, polyethyleneglycol; PFD, photon flux density; Pn, net photosynthetic rate; PSI, photosystem I; PSII, photosystem II; POD, peroxidase; REL, relative electrolyte leakage; ROS, reactive oxygen species; RWC, relative water content; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; $^1Chl^*$, singlet-excited chlorophyll; $^1O_2$, singlet oxygen; SOD, superoxide dismutase; *SlLUT1*, *Solanum lycopersium* carotenoid epsilon-ring hydroxylase gene; TEM, transmission electron microscopy; $^3Chl^*$, triplet chlorophyll; VDE, violaxanthin de-epoxidase; WT, wild-type; ZE, zeaxanthin epoxidase

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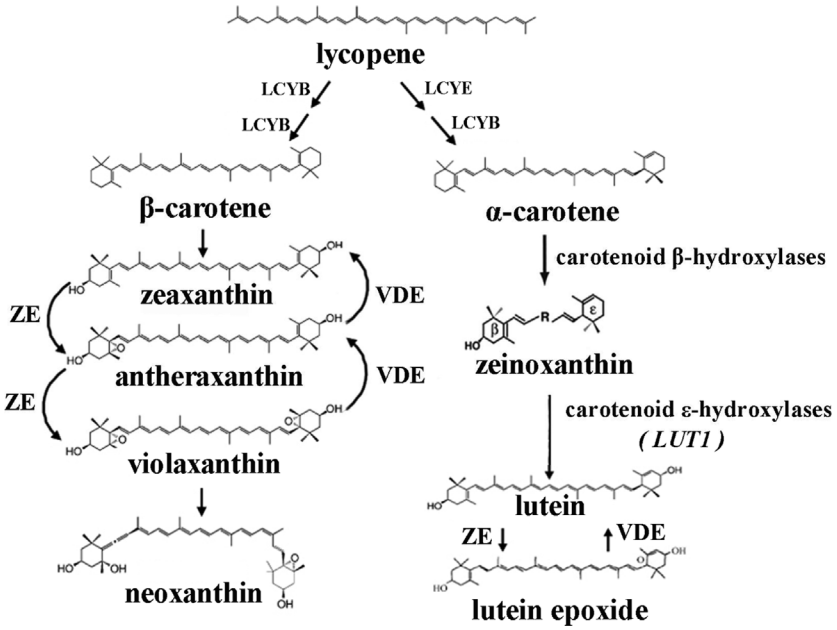

Fig. 1. Carotenoid biosynthetic pathway in plants. Reactions of the violaxanthin cycle (left) and the lutein-epoxide cycle (right) in carotenoid biosynthesis pathway. LCYE, lycopene ε-cycle; LCYB, lycopene β-cycle; VDE, violaxanthin de-epoxidase; ZE, zeaxanthin epoxidase.

Moreover, carotenoids decrease the congestion of ROS through the thermal dissipation of excess light energy in the state of singlet-excited chlorophyll ($^1Chl^*$).

Carotenoids are a family of terpenoid compounds that play vital roles in photosystem structure, light harvesting, and photoprotection. Lutein (3*R, 3′R-β, ε*-carotene-3, 3′-diol) is the most productive xanthophyll in the plant photosynthetic apparatus, and it serves a significant function in PSII assembly (Li et al., 2001). Lutein has several important functions: (1) stabilization of antenna proteins, (2) light harvesting (transfer of excitation energy to Chl), and (3) quenching of $^3Chl^*$ states. Moreover, lutein may also be involved in the quenching of $^1Chl^*$.

The synthesis of lutein from lycopene requires four distinct enzymatic reactions that involve β- and ε-ring cyclizations and the hydroxylation of each ring (Kim and Dellapenna, 2006). Carotenoid ε-hydroxylase, which is encoded by *LUT1*, is a key enzyme of the lutein biosynthetic pathway in plants (Fig. 1). Numerous studies have investigated *LUT1*, and it has been identified as a member of the cytochrome P450 monooxygenase family (Li et al., 2003). Functional analysis of the *LUT1* gene is crucial for understanding lutein biosynthesis and the regulation of ring hydroxylations. It has been found that *LUT1* is required for carotenoid ε-ring hydroxylation activity (Li et al., 2004). Adriana et al. (2011) demonstrated that the hydroxylation of α-carotene to lutein occurs via the CYP97A29-mediated β-hydroxylation of α-carotene to zeinoxanthin followed by the CYP97C11-mediated ε-ring hydroxylation of zeinoxanthin to lutein. Chang et al. (2015) isolated the *CYPC19* gene, a putative heme-containing carotenoid ε-hydroxylase, from maize endosperm. However, the functions of CYPC19 in vegetables, such as tomato, have rarely been reported. Tomato is a very important vegetable that is highly sensitive to abiotic stresses. Given its possible relationship with drought stress, we focused on the expression of the tomato carotenoid ε-ring hydroxylase gene (*SlLUT1*). We studied its physiological function with regard to the amount of accumulated lutein in transgenic tobacco plants. *SlLUT1* overexpression significantly increased leaf lutein content and alleviated drought-stress-induced photoinhibition and photooxidation. Thus, we suggest that *SlLUT1* overexpression could promote drought resistance in transgenic tobacco.

2. Materials and methods

2.1. Plant growth conditions and treatment

Plants used in this study included WT tomato (*Solanum lycopersium* cv. Zhongshu 6), WT tobacco (*Nicotiana tabacum* cv NC 89), T_2 transgenic tobacco plants (Zhou et al., 2013), and *Arabidopsis thaliana* (Colombia). The plants were grown under conditions previously described by Zhang et al. (2016).

For drought treatment, eight-week-old WT and transgenic tobacco were treated with 20% polyethyleneglycol (PEG-6000) solution for four days. Then, a photo was taken of the plants to document their phenotype. Simultaneously, functional leaves of the plants were cut and collected. The leaves were soaked in liquid nitrogen instantly and stored at −80 °C until use.

2.2. Histochemical assay

An approximately 2.0-kb-long fragment of the SlLUT1 promoter was inserted in the expression vector pZP121 with luciferase (*LUC*) gene. The carrier with the *SlLUT1* promoter-*LUC* reporter was transformed into *Arabidopsis* via the *Agrobacterium*-mediated method. Ten-day-old transgenic *Arabidopsis* plants were placed in MS plates containing no mannitol or with 200 mM mannitol for 0, 3, 6, 9 and 12 h to analyze the *LUC* expression pattern under drought treatment. Plants were visualized using IVIS Lumina II.

2.3. Quantitative RT-PCR assay

The Bio-Rad CFX96TM Real-Time PCR Detection System (USA) was utilized to implement quantitative real-time PCR (qRT-PCR) assays with Super Real Premix Plus (TIANGEN, Beijing, China). Transcript levels of *NtUbiquitin* (GenBank U66264.1) and *EF*-1α (GenBank Accession No. LOC544055) were used as a control. qRT-PCR analyses were performed with three biological replicates. The primers used in this study are listed in Table 1.

2.4. Pigment analysis

Lutein was extracted from tobacco with acetone, and pigment