Journal of Plant Physiology 209 (2017) 31–41

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Overexpression of tomato *SlGGP-LIKE* gene improves tobacco tolerance to methyl viologen-mediated oxidative stress

Dong-Yue Yang[1], Na-Na Ma[1], Kun-Yang Zhuang, Shao-Bo Zhu, Zhong-Ming Liu, Xing-Hong Yang*

College of Life Science, State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, Shandong 271018, PR China

ARTICLE INFO

Article history:
Received 11 April 2016
Received in revised form 25 October 2016
Accepted 28 October 2016
Available online 25 November 2016

Keywords:
Tobacco
AsA
ROS
GGP
Methyl viologen
Violaxanthin de-epoxidase

ABSTRACT

Ascorbate (AsA) is very important in scavenging reactive oxygen species in plants. AsA can reduce photoinhibition by xanthophyll cycle to dissipate excess excitation energy. GGP is an important enzyme in AsA biosynthesis pathway in higher plants. In this study, we cloned a gene, *SlGGP-LIKE*, that has the same function but different sequence compared with *SlGGP*. The function of *SlGGP-LIKE* gene in response to oxidative stress was investigated using transgenic tobacco plants overexpressed *SlGGP-LIKE* under methyl viologen treatment. After oxidative stress treatment, transgenic tobacco lines exhibited higher levels of reduced AsA content and APX activity than WT plants. Under oxidative stress, transgenic tobacco plants accumulated less ROS and exhibited lower degrees of REC and MDA. Consequently, relatively higher levels of Pn, Fv/Fm, de-epoxidation status of xanthophyll cycle and D1 protein were maintained in transgenic tobacco plants. Hence, overexpression of *SlGGP-LIKE* gene enhances AsA biosynthesis and can alleviate the photoinhibition of PSII under oxidative stress.

1. Introduction

In plants, ROS are generated during the metabolic process in cells, and the generation and scavenging of ROS maintain dynamic balance. When plants subjected to environmental stresses, the accumulation of ROS is rapid and plants have formed mechanisms to detoxify ROS. Ascorbate (AsA) is an essential material in protecting plants from damage of excess ROS that generated during abiotic or biotic stresses (Conklin, 2001; Foyer and Noctor, 2005; Müller-Moulé et al., 2004; Laing et al., 2015). AsA can reduce photoinhibition by the xanthophyll cycle which can promote the conversion of violaxanthin (V) to zeaxanthin (Z) to eliminate excess light energy (Chen and Gallie, 2006; Gallie, 2013; Miyaji et al., 2015). In addition, AsA can also donate electron in the elimination of H_2O_2 catalyzed by APX. In particular, chloroplasts use AsA to eliminate the ROS produced by photosystem II (PSII) for the synthesis of NADPH in the stroma. AsA is drawn into the elimination of excess energy via heat dissipation in the xanthophyll cycle acting as a coenzyme.

The enzyme GGP (GGP: EC 2.7.7.69) is vital important in the Smirnoff-Wheeler pathway (L-galactose pathway) for AsA synthesis (Wheeler et al., 1998). Overexpression of GGP genes can increase the AsA concentrations in plants (Smirnoff, 2000; Laing et al., 2007; Bulley et al., 2009, 2012), thereby indicating that it may balance fluxes between AsA and GDP-L-fucose function as a regulatory role. The *Arabidopsis thaliana vtc2* mutant, which encodes GGP in Arabidopsis, has less AsA content (Linster et al., 2007; Miyaji et al., 2015). Many results indicate that manipulating the gene that regulates the expression of AsA affects the stress tolerance of plants (Kwon et al., 2002; Lim et al., 2007; Li et al., 2010; Wang et al., 2013a,b).

GGP exists in majority of plants, whereas tomato has two GGP genes (*SlGGP* and *SlGGP-LIKE*) which are present in chromosomes 6 and 2, respectively. GGP is vital to plants to maintain the AsA pool, and this gene has been reported in tomato and tobacco (Laing et al., 2007, 2015; Bulley et al., 2009; Wang et al., 2013a,b). However, the

Abbreviations: A, antheraxanthin; Fv/Fm, the maximal photochemical efficiency of PSII; GDP, guanosine 5′-diphosphate; GFP, green fluorescent protein; GGP, GDP-L-galactose phosphorylase; HPLC, high performance liquid chromatography; MDA, malondialdehyde; MV, methyl viologen; PBS, phosphate buffer saline; PSII, photosystem II; $O_2^{\bullet-}$, superoxide anion radical; Pn, net photosynthetic rate; PFD, photon flux density; PVDF, polyvinylidene fluoride; REC, relative electronic conductance; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; V, violaxanthin; VDE, violaxanthin de-epoxidase; WT, wild-type; Z, zeaxanthin; ZEP, zeaxanthin epoxidase.

* Corresponding author at: State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, 61 Dai Zong Street, Tai'an, Shandong 271018, PR China.
E-mail address: xhyang@sdau.edu.cn (X.-H. Yang).
[1] Both these authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jplph.2016.10.013
0176-1617/

function of *GGP-LIKE* is less reported compared with that of *GGP* gene.

In this study, a tomato gene *SlGGP-LIKE* which encoded GDP-L-galactose phosphorylase and located in nucleus, was cloned and transformed into tobacco. The expression of *SlGGP-LIKE* can be induced by chilling stress (4 °C), high salinity, oxidative stress, high light, salicylic acid, methyl jasmonate, and pathogen attack. In our experiments, we used MV to simulate oxidative stress. Under MV treatment, transgenic plants exhibited higher levels of reduced AsA concentration and activity of APX than WT plants, and thus detected less ROS content in transgenic plants. GGP-LIKE is also important in improving the de-epoxidation status of xanthophyll cycle by increasing AsA content under oxidative stress.

2. Theory

ROS are produced inevitably in cell metabolism such as respiration and photosynthetic electronic transmission and will attack the biomacromolecule such as protein, nucleic acid, lipids and induce oxidative damage to cell and tissues if they could not be scavenged efficiently under environmental stresses. In order to analyze the roles of *SlGGP-LIKE* gene under oxidative stress, we obtained *SlGGP-LIKE*-overexpressed transgenic tobacco plants and measured the changes of Pn, REC and MDA in WT and transgenic lines under oxidative stress. The transgenic plants exhibited an elevated endurance against oxidative stress compared with WT plants after MV treatment.

3. Materials and methods

3.1. Plant materials, growth, and treatments

Wild tomato cultivar (*Solanum lycopersium* cv. Zhongshu 6) was used as plant materials.

Seeds were sown on MS agar medium after sterilized and incubated in an incubator at 25 °C (light 16 h/dark 8 h) for 8 d. Young seedlings were planted in sterilized soil and grown in a greenhouse. Eight-week-old tomato seedlings were treated with various stresses. For chilling treatment, the plants were exposed to 4 °C in the incubator. For high light treatment, the plants were exposed to 2000 μmol m^{-2} s^{-1} light from daylight-type microwave sulfur lamps (MSL-1000; Youhe, Ningbo, China). Salinity stress was performed by immersing the whole plants in 200 mM NaCl solution. The plant leaves were sprayed with 100 μM MeJA, 100 μM SA to simulate hormone treatments. For oxidative treatment, the plants were sprayed with 100 μM MV. For pathogen treatment, *Pseudomonas syringae* pv. tomato DC3000 suspensions [1×10^8 cfu (colony forming units) ml^{-1}] were used to infect the leaves. The treated leaves were harvested, frozen in liquid nitrogen, and stored at −80 °C.

3.2. Subcellular localization of SlGGP-LIKE

The full-length cDNA of *SlGGP-LIKE* was cloned and two DNA constructs (p35S-*GFP* and p35S-*SlGGP-LIKE-GFP*) were obtained. *Nicotiana benthamiana* seedlings were injected with the above two constructs and epidermis was examined by dual channel confocal microscopy (LSM510 META; Zeiss, Germany).

3.3. Obtainment of transgenic tobacco

The full-length of *SlGGP-LIKE* gene was cloned with primers GGPL-F and GPL-R. Subsequently, the PCR product was subcloned into the botany expression vector pBI121 with 35S promoter upstream to construct overexpression constructs (pBI-*SlGGP-LIKE*). The recombinant plasmids (pBI121-*SlGGP-LIKE*) were transferred into tobacco plants using *Agrobacterium* strain EHA105-mediated cotyledon infiltration. After a few weeks, 14 independent kanamycin-resistant lines were achieved. These self-pollinated transgenic tobacco plants (T_0 generation) then produce seeds of T_1 generation. The seeds of T_1 generation were planted on Murashige–Skoog (MS) agar medium at 25 °C, a 16-h light/8-h dark photoperiod and photon flux density (PFD) of 600 μmol m^{-2} s^{-1}. After two weeks, seedlings were cultivated into vermiculite and grown in the greenhouse.

3.4. Plant growth

WT seeds of tobacco (*Nicotiana tabacum* L. cv. NC89) and tomato (*Solanum lycopersicum* cv. Zhongshu 6) were germinated on MS agar media, whereas transgenic tobacco seeds were germinated on MS agar media with kanamycin (50 μg mL^{-1}) in tissue culture vessels for a week at 25 °C, then the plants were moved into vermiculite and grown in greenhouse. Eight-week-old tomato and eight-week-old transgenic tobacco plants were applied to oxidative stress and qRT-PCR assays.

3.5. Methyl viologen treatment

Plants were sprayed with 100 μM of MV evenly and deionized water as control, and then the plants were placed into a chamber with PFD of 200 μmol m^{-2} s^{-1} for 24 h continuously. Detached leaves were put into liquid nitrogen quickly and stored at −80 °C until use.

3.6. Germination analysis

To analyze the influence of MV on seed germination, seeds of WT and transgenic tobacco were cultivated on MS agar media with different concentrations of MV (0, 5 and 10 μmol L^{-1}) in Petri dishes. The seeds were subsequently placed in a chamber (GXZ-260C) with PFD of 200 μmol m^{-2} s^{-1}, with a constant temperature of 25 °C and a 16-h light/8-h dark photoperiod for about two weeks. Photographs were then taken, and the germination rate was calculated.

3.7. Determination of reduced, oxidized and total AsA

The reduced AsA (AsA) contents, oxidized AsA (DHA) contents and total ascorbates (AsA + DHA) content were determined according to Li et al. (2010) with a little modification. 0.5 g detached leaves were ground into homogenized with 2 mL 6% TCA and transferred to a centrifugal tube. After centrifuged at 12000 × *g* for 10 min at 4 °C, the supernatant was used to measure the content of AsA and oxidized ascorbic acid (DHA), AsA reaction solution including 0.2 mL extract (blank to 6% TCA instead), 0.6 mL 0.2 M PBS (pH 7.4), 0.2 mL ddH_2O, 1 mL 6% TCA, 0.8 mL 42% H_3PO_4, 0.8 mL 4% 2,2′-bipyridine and 0.4 mL 3% $FeCl_3$. The total AsA was (AsA + DHA) measured with 0.2 mL 10 mM dithiothreitol and 0.4 mL 0.2 M PBS (pH 7.4) instead of 0.6 mL 0.2 M PBS above, and the reaction solution bated 1 h in 42 °C. Absorbance was measured at 525 nm. The content of DHA can be measured by the difference of total AsA content and without DTT AsA content.

3.8. Measurement of H_2O_2 and $O_2^{\bullet -}$

About 0.5 g leaf samples were homogenised with 3 mL of pre-cooled acetone to measure H_2O_2 concentration. After centrifugation at 2000 × *g* for 10 min, 0.1 mL of 5% titanium sulphate and 0.2 mL of $NH_3 \cdot H_2O$ were added into the separated 1 mL supernatant. The mixture was centrifuged again and washed 3–5 times with 1 mL of acetone. Finally, the precipitate was dissolved in